

Quantity and specialisation matter: Effects of quantitative and qualitative variation in willow chemistry on resource preference in leaf-chewing insects

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Abstract

1. Plants produce multiple specialised metabolites to defend themselves against insect herbivores. Phytochemical diversity plays important roles in plant–insect interactions, but specific roles of its various dimensions are poorly known. Interspecific chemical β -diversity represents variation in presence of species-specific metabolites or quantitative variation in concentrations of metabolites common to several plant species.
2. We hypothesised that qualitative and quantitative variation in plant chemistry can have differential effects on herbivores from various insect orders.
3. We linked phytochemical variation in willow salicylates (Salicaceae-specific metabolites) and flavonoids (widespread metabolites) to a standardised distance-based specialisation index (DSI*) in three orders of leaf-chewing insects: sawfly larvae, beetles, and caterpillars.
4. In beetles, average DSI* accounting for host chemical β -diversity did not differ from DSI* disregarding host chemistry. Levels of chemical specialisation did not differ among beetle species feeding only on Salicaceae and those using other plant families, suggesting that both can overcome willow chemistry by alternative physiological or behavioural adaptations. Contrastingly, sawflies and caterpillars responded to willow chemistry, with their DSI* corresponding mainly to quantitative differences in willow metabolites. The DSI* accounting for salicylates did not differ from the one accounting for flavonoids in either of the two orders.
5. Our results suggest that β -diversity in plant chemistry has differential effects on insect herbivores depending on their order and chemical β -diversity measurement used. Our results emphasise the importance of quantitative variation in plant chemical composition, suggesting that it does not always have to be rare or species-specific metabolites that drive host-choice of leaf-chewing insects.

KEYWORDS

beta-diversity, flavonoids, generalists, herbivory, leaf-chewers, phytochemistry, salicylates, *Salix*, specialists

INTRODUCTION

Highly diverse plant metabolites mediate plant interactions with other organisms and help them withstand abiotic stress (Wetzel & Whitehead, 2020). Ehrlich and Raven (1964) proposed that insect herbivores are an especially strong force driving the genesis of novel and lineage-specific metabolites, giving rise to the bewildering diversity of specialised metabolites we observe. Various hypotheses have attempted to investigate how ecological processes could explain the high variation in phytochemicals and how plants benefit from high phytochemical diversity in response to herbivory (Glassmire et al., 2020; Wetzel & Whitehead, 2020). However, the functional roles of phytochemical diversity and their effect on insect communities are still not fully understood (Wetzel & Whitehead, 2020). This is partly due to the complex interplay between herbivory and the many dimensions of plant chemical diversity (Glassmire et al., 2020; Volf et al., 2019; Wetzel & Whitehead, 2020). For example, while phytochemical α -diversity represents the richness of compounds within a plant individual, species, or lineage, phytochemical β -diversity can be measured as qualitative or quantitative differences between plants (Wetzel & Whitehead, 2020). Phytochemical β -diversity can thus represent both the variation in presence of lineage-specific metabolites and variation in the concentration of metabolites common across plants.

Most insect herbivores are phylogenetically and chemically conservative in their food choice and prefer feeding on closely related or chemically similar hosts (Endara et al., 2017). Host-plant chemical similarity thus typically correlates with similarity in herbivore communities (Coley et al., 1985; Volf, Julkunen-Tiitto, et al., 2015). Chemical β -diversity seems to play an especially prominent role in large speciose plant genera that include multiple species growing in sympatry. Previous studies have shown low phylogenetic signal and high divergence in the defences of multiple tropical genera such as *Bursera* Jacq. ex L., *Inga* Mill., *Ficus* Tourn. ex L., *Piper* L., or *Psychotria* L. (Endara et al., 2017; Volf et al., 2018; Richards et al., 2015; Sedio et al. 2017). The resulting high inter-specific chemical β -diversity helps plants from these genera avoid sharing herbivores with their sympatric congeners and can reduce the damage they suffered from herbivory (Becerra, 2007; Glassmire et al., 2020; Volf et al., 2019).

Common metabolites include chemical groups that are widespread among plants, such as various polyphenols (Bennett & Wallsgrave, 1994; Salminen & Karonen, 2011). Other metabolites, in contrast, are restricted to individual plant lineages (Agrawal & Fishbein, 2006). Several of these metabolites are toxic and deterrent to generalist herbivores, even in relatively low concentrations (Coley et al., 1985). For example, qualitative differences in the alkaloid content among deciduous trees correlate with the feeding preference of the highly polyphagous *Lymantria dispar* L. (Barbosa & Krischik, 1987). In contrast, specialised insect herbivores have repeatedly evolved adaptations that allow them to cope with lineage-specific defences of their hosts (van der Meijden, 1996; Ruuhola, 2001; Ruuhola et al., 2001, Gleadow & Woodrow, 2002; Dyer et al., 2004; Roslin & Salminen, 2008). Specialised herbivores can thus sometimes tolerate high concentration of such metabolites, use them for host-recognition

as feeding or oviposition cues, or sequester them and turn them into their own defence against predators (Boeckler et al., 2011; Hardy et al., 2020).

With these adaptations to specialised plant metabolites by insect herbivores, the varying effects of chemical β -diversity are thus likely affected by the level of specialisation of herbivores, the type of metabolites involved, and the qualitative or quantitative differences of plant metabolites in play. For example, the presence or absence of individual glucosinolates is what seems to drive herbivore communities associated with wild cabbage (Ratzka et al., 2002). In contrast, the quantitative differences in the overall content and diversity of alkaloids drive abundance of specialised herbivores in *Ficus* (Volf et al., 2018).

Willows (*Salix* L.) are a diverse genus including 450 species belonging to the Salicaceae family (Fang, 1987; Skvortsov, 1999). The vast majority of willow species occur in temperate regions of the northern hemisphere, where they harbour a high diversity of insect herbivores, thus representing a key-stone plant genus for insect herbivores (Narango et al., 2020). Therefore, it is an excellent parallel to species-rich tropical genera (i.e. *Ficus*, *Protium*, or *Inga*; Volf et al., 2018; Salazar et al., 2016; Becerra, 2007), in which the effects of chemical β -diversity on diverse insect communities have been typically tested. Willows possess diverse chemical defences, based primarily on various phenolics. These include flavonoids and tannins that are widespread among plants (Ruuhola et al., 2001; Volf et al. 2015,b). Additionally, willows also produce salicylates that are largely specific to the Salicaceae family and have pronounced effects on insect herbivores that are associated with willows (Julkunen-Tiitto, 1989).

Salicylates typically have negative effects on generalist herbivores. These effects include deterring generalist herbivores, such as various beetles and caterpillars, from feeding, slowing their growth rates, or increasing their mortality (Denno et al., 1990). In contrast, some specialised leaf-beetles can use salicylates as feeding cues (Kolehmainen et al., 1995) and their larvae grow faster on salicylate-rich willows (Denno et al., 1990). Some of these beetles can even sequester salicylates and produce salicylaldehyde, which their larvae use as a defence against invertebrate predators (Rowell-Rahier & Pasteels, 1986). Faster growth rates on salicylate hosts have been also recorded in sawflies that probably use salicylates as oviposition cues (Lahtinen et al., 2005).

Here, we use eight willow species, two willow hybrids, and one poplar to test for the effects of the chemical β -diversity of Salicaceae on different insect orders. We assess whether differences in lineage-specific versus widespread or abundant versus rare metabolites have a greater effect on specialisation of insect herbivores within willows. To do so, we focus on salicylates as a lineage-specific defence and flavonoids as a widespread defence. We use quantitative and qualitative phytochemical β -diversity metrics to shift emphasis between differences in relative concentrations and presence of different sets of metabolites in different hosts. To test our hypotheses, we use three orders of leaf-chewing insects: (i) Hymenoptera (sawflies) that include mainly highly specialised species that predominantly feed only on willows and poplars, (ii) Coleoptera (beetles) that include a mix of generalists that feed on other plant families and willow specialists, including



sequestering leaf beetles; and (iii) Lepidoptera (caterpillars) that include many generalists which feed on many other plant families than Salicaceae. We aim to determine whether the chemical β -diversity metrics based on qualitative (presence-absence) differences of all metabolites, or quantitative (abundance) differences of all metabolites, flavonoids, and salicylates drive herbivore host-plant specialisation. Generally, we expect that herbivore specialisation will be driven more strongly by the effects of chemical β -diversity in Salicaceae-specific salicylates than the chemical β -diversity in widespread flavonoids. Additionally, we expect beetle and sawfly host-plant preference to be affected mainly by quantitative differences in salicylates, because some of them use salicylates as feeding or oviposition cues or sequester them to produce anti-predator defences. Conversely, we hypothesise that caterpillar host-plant preference will be mainly driven by qualitative differences in salicylates that can probably deter them even in small concentrations.

METHODS AND MATERIALS

Background data

We based our analyses on previously published data on willow chemistry and insect herbivores that we extracted from Volf et al. (2015a, 2015b). The original study was carried out within a 10 × 10 km area composed of lowland wet meadows, or forest margins in South Bohemia, Czech Republic (48°51'58"–48°59'45"N, 14°26'20"–14°35'48"E, ~500 m a.s.l.) in 2008–2011 during the growing season (end of April to the end of September). All plants sampled represented mature trees and shrubs. Shaded or heavily herbivorised plants were not sampled to avoid possible effects of these factors on their chemistry and insect communities. The dataset comprises information on eight willow species (*Salix aurita* L., *S. caprea* L., *S. cinerea* L., *S. euxina* Belyaeva *S. pentandra* L., *S. purpurea* L., *S. rosmarinifolia* L., and *S. viminalis* L.), two willow hybrids (*S. alba* × *S. euxina* and *S. purpurea* × *S. viminalis*), and one poplar (*Populus tremula* L.).

Two to seven individuals per species (48 individual plants in total) were sampled for leaves in 2010 at the beginning of June when insect density in the study area reaches its peak to analyse salicylates and flavonoids. We sampled well-separated individuals and spread our sampling across several sites within the studied area to get representative samples and to avoid sampling clonal plants as far as possible. The leaf samples were put in silica gel immediately after collection in the field. Then, they were air-dried in an oven at 40°C overnight or until completely dried. Contents of individual salicylates and flavonoids (mg g^{-1}) were analysed from 5 to 9 mg of homogenised leaf material. The metabolites were extracted with methanol following methods from Nybakken et al. (2012). The extracts were dried and stored at –20°C. Dried samples were re-dissolved in 600 μl methanol : water (1:1). Twenty microliters of re-dissolved samples were used for high-performance liquid chromatography (HPLC) for analysis of salicylates and flavonoids following Nybakken et al. (2012). Compounds were separated using a Zorbax SBC18 (4.6 × 60 mm) HPLC column

(Agilent Technologies, Waldbronn, Germany) using a water/methanol gradient (Julkunen-Tiitto & Sorsa, 2001). Salicylate and flavonoid contents were measured based on the absorbance at 270 nm and 320 nm, respectively. Retention times and spectra compared to standards were used to identify the compounds. Altogether, 136 compounds were detected, which consisted of 28 salicylates and 108 flavonoids across all sampled trees in this study (Volf et al. 2015b). *Salix rosmarinifolia* was richest in salicylates (average total = $169 \pm 42.0 \text{ mg g}^{-1}$, number of salicylates detected = 14) while *S. aurita*, *S. caprea*, *S. cinera*, and *S. viminalis* contained no salicylates. The distribution of flavonoids was more equal among the studied plant species (Table S1).

The insects were sampled by beating and manual search in ~1-week intervals in April to September every year between 2008 and 2011. The studied host species included trees and shrubs with various canopy size. We thus used sampling effort in minutes to standardise the insect abundance across the studied plant species (Table S2). Insects at immature stages were reared to adults for identification purposes and dead larvae were morphotyped based on photographs. The sampled caterpillars and sawflies were experimentally verified to feed on the sampled tree species with feeding trials. Beetles were verified to feed on the sampled plant species through field observation or referencing available literature on their host records. In total, 9106 individual insects were sampled consisting of 126 insect species (Table S2). Details of the methods described above are further explained in the study by (Volf et al. 2015a, 2015b).

Statistical analyses

In order to tease apart which aspects of chemical β -diversity best predict herbivore host-plant preferences (based on the abundance of herbivores on sampled tree species), we measured host specialisation using five different ways to account for chemical β -diversity: (i) a null metric that accounts for host-specialisation disregarding host chemistry, (ii) presence or absence of plant metabolites using Jaccard's similarity index, (iii) abundance of all plant metabolites using Bray–Curtis' similarity index, (iv) a model that only accounts for the abundance of salicylates using Bray–Curtis' similarity index, and lastly, and (v) a model that only accounts for the abundance of flavonoids using Bray–Curtis' similarity index. We computed the similarity indices using the 'vegan' package (Oksanen et al., 2018).

Specialisation was measured using the standardised distance-based specialisation index (DSI*) (Jorge et al., 2014, 2017), using the five β -diversity approaches listed above. This index takes resource similarity – in our case, chemical similarity – and resource availabilities and sampling effort into account. Therefore, DSI* reflects the specialisation of insect herbivores more mechanistically than traditional indices and allows for robust comparisons between datasets of varying abundances, communities with different ranges of resource species richness (Jorge et al., 2014, 2017). DSI* ranges between –1, for maximum generalisation, and 1, for strict monophagous species. DSI* was calculated separately for each herbivore species and chemical β -diversity metric.

With these five chemical β -diversity metrics mentioned above, we measured specialisation and compared them between the three insect orders and feeding preferences considered in this study system using linear mixed models (LMMs) generated using the 'lme4' package (Bates et al., 2015). Insect order, herbivore feeding preference, and the type of β -diversity metric were used as fixed effects, while herbivore species was used as the random effect. The square root of the insect herbivore abundances was taken as weights in the models, so that more abundant herbivores contribute more to the model. Moreover, it also prevents herbivores of low abundances (and thus less reliable records) to take over the effects of the model. This approach allowed us to focus exclusively on lineage specific versus widespread metabolites and consider quantitative or qualitative aspects of functional chemical β -diversity. To select the best model explaining variation in DSI*, we determined whether insect order, β -diversity distance metric, or preference for Salicaceae, may affect herbivore DSI* by using the corrected Akaike information criterion (AICc). We defined preference for Salicaceae using three categories based on the literature (Lacourt, 1999; Macek et al., 2007, 2008, 2012; Pasteels et al., 1984; Rowell-Rahier & Pasteels, 1986; Smreczynski, 1966, 1972; Warchalowski, 2003): (1) sequestering specialists, referring to species which exclusively feed on Salicaceae and sequester salicylates; (2) Salicaceae specialists, which include all species feeding exclusively on Salicaceae but are not known to sequester salicylates; and (3) generalists, including all herbivore taxa which feed on plant families beyond Salicaceae. Based on the categories defined above, 41% of the sampled insect herbivore species were generalists, 56% are specialists that do not sequester salicylates, and 3% are known to sequester salicylates. All statistical analyses were run on R v. 4.0.2 (R Core Team, 2020).

RESULTS

The three insect orders differed in their interactions with the studied hosts, with several caterpillar and beetle species interacting with a wider range of willow species compared to sawflies (Figure 1). The best model explaining variation in DSI* included insect order, chemical β -diversity metric, and the interaction between insect order and chemical β -diversity metric (Table S3), with no effect of preference for Salicaceae as defined in our methods. Our results confirmed pronounced differences in DSI* between the three insect orders (Figure 2, Table S4). Sawflies exhibited the highest mean DSI* in both host specialisation and various definitions of chemical β -diversity specialisation. They were followed by beetles, then caterpillars (Table S4). There was a significant difference between sawflies and caterpillars across all host and chemical β -diversity specialisation metrics. Likewise, beetles and sawflies significantly differed from each other across all host and chemical β -diversity specialisation definitions. However, the differences between beetles and caterpillars were non-significant when we accounted for differences in host chemistry (Table S5).

Average beetle DSI* did not significantly differ among the chemical β -diversity specialisation definitions (Figure 2, Table S4). Interestingly, the beetle species with the lowest DSI* showed higher

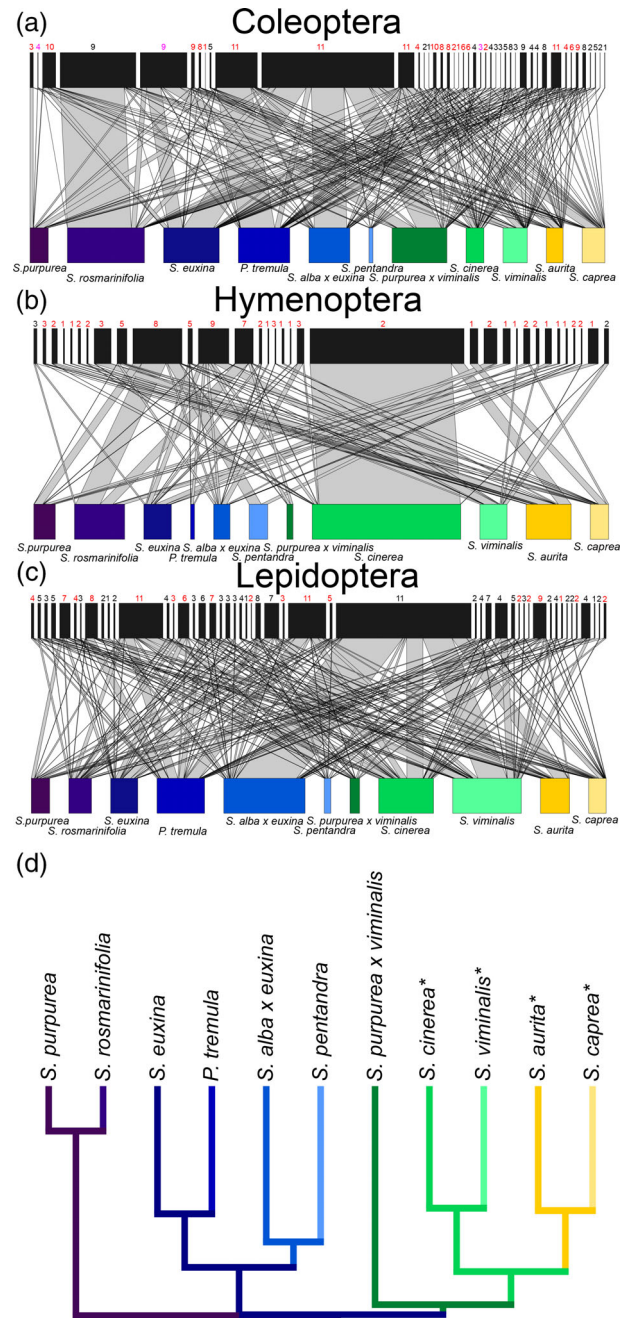


FIGURE 1 Food webs showing associations of beetles (Coleoptera, a), sawflies (Hymenoptera, b), and caterpillars (Lepidoptera, c) to the studied Salicaceae species. The numbers above the insect herbivore blocks (black) represent the number of interactions an insect herbivore is making with each plant species. Black numbers indicate generalists, specialists are in red, and sequestering specialists are in purple. Colour coding of the plant blocks reflect their chemical similarity as measured by Bray-Curtis index (d). The data on insects and chemistry of *S. aurita*, *S. caprea*, *S. cinerea*, *S. euxina*, *S. pentandra*, *Salix purpurea*, *S. rosmarinifolia*, *S. viminalis*, *S. alba x euxina*, *S. purpurea x viminalis*, and *Populus tremula* were taken from Volf et al. (2015, 2015b). Asterisks (*) indicate willow species that do not produce salicylates

host specialisation than chemical β -diversity specialisation (Figure 2), a trend that was directly opposite to the one in sawflies and caterpillars. Both sawfly and caterpillar DSI* were affected mainly by quantitative

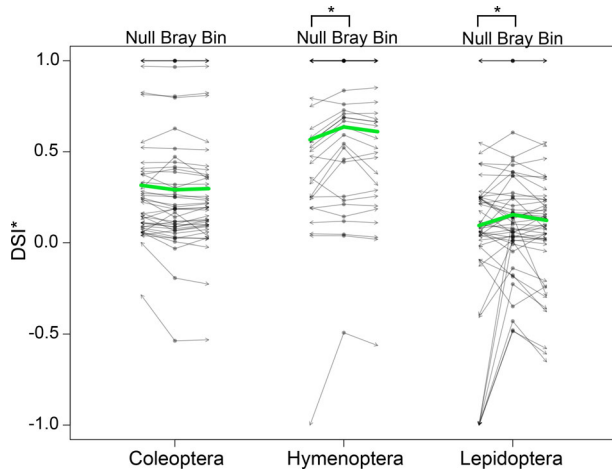


FIGURE 2 Distance-based specialisation index (DSI*) of beetles (Coleoptera), sawflies (Hymenoptera), and caterpillars (Lepidoptera) accounting for host specialisation (null), chemical β -diversity specialisation in quantitative similarity in host chemistry as measured with Bray–Curtis similarity index (Bray), and chemical β -diversity specialisation in qualitative similarity in their chemistry as measured with binary Jaccard similarity index (bin). Each point represents an insect herbivore species. Asterisks (*) indicate significant difference between specialisation indices within the three insect orders. Green lines indicate mean DSI*

differences in willow metabolites (Table S4). Sawflies exhibited a higher DSI* when accounting for abundance of salicylates than when accounting for abundance of flavonoids. In contrast, caterpillars showed somewhat higher DSI* when accounting for abundance of flavonoids than when accounting for abundance of salicylates. However, these differences in DSI were not significant in either insect order (Table S4).

DISCUSSION

Plant chemical diversity has pronounced effects on herbivorous insects (Richards et al., 2015; Salazar et al., 2016). These effects typically depend on the dimension of chemical diversity in play (Wetzel & Whitehead, 2020). Here, we investigated the effects of chemical β -diversity between host species on 3 insect orders associated with 10 willow and 1 poplar species. We measured their chemical β -diversity specialisation using DSI* with respect to quantitative and qualitative chemical differences between the hosts as well as to differences in widespread (flavonoids) and specialised (salicylates) chemical defences. In line with previous studies, our results suggest that the effect of specialised metabolites on insect herbivores is linked to their level of dietary specialisation (Ali & Agrawal, 2012; Volf, Hrcek, et al., 2015). However, this trend is not completely trivial as even herbivores with relatively high host specialisation do not necessarily have to show high chemical β -diversity specialisation at host genus or family level.

Beetle specialisation did not depend on any chemical β -diversity specialisation considered. This suggests that beetle host-plant

preference was not very strongly affected by the willow chemistry we measured. Beetles that feed on willows and poplars exhibit diverse life-histories, including many specialised species that feed predominantly on Salicaceae and comprise a substantial part of the dataset we analysed (Topp et al., 2002; Volf, Julkunen-Tiitto, et al., 2015). Some of these species can use specialised metabolites of their Salicaceae hosts as feeding cues (Kolehmainen et al., 1995). The most specialised species can even use willow and poplar salicylates for their own benefit. For example, leaf beetle *Phratora vitellinae* (Coleoptera, Chrysomelidae) that was the third most abundant species in the dataset (947 individuals) can sequester salicylates and its larvae excrete salicylaldehyde from their abdominal glands to repel invertebrate predators (Pasteels et al., 1984). Its larvae also seem to grow faster on salicylate-rich species (Denno et al., 1990) and are able to utilise multiple salicylate-rich Salicaceae species as their hosts (Volf, Hrcek, et al., 2015; Volf, Julkunen-Tiitto, et al., 2015). Even more abundant species was *Crepidodera aurata* Marsham (Coleoptera, Chrysomelidae, commonly known as the willow flea beetle), a Salicaceae specialist, that fed on all hosts in the study and comprised ~35% (3192 individuals) of all herbivores we sampled. The exact mechanisms how it copes with various chemistry of its Salicaceae hosts is unknown, but related flea beetle species are known to detoxify and sequester metabolites of their Brassicaceae hosts (Gikonyo et al., 2019). If similar mechanisms are present in *C. aurata*, it could explain why they are so abundant and can feed on multiple Salicaceae species disregarding β -diversity in their chemistry.

In addition to specialists, willows and poplars also harbour high diversity of beetles that feed on hosts outside of Salicaceae (Topp et al., 2002; Volf, Hrcek, et al., 2015). Interestingly, the beetle species with the lowest DSI* had an even lower specialisation in the metrics accounting for chemical β -diversity than the null metric using only number of hosts. Many polyphagous herbivores switch between chemically dissimilar hosts as mixing their diet helps them avoid negative effects of host chemistry (Pasteels et al., 1984). This strategy can be especially successful in case of mobile herbivores, such as adult beetles, and could explain the trend we observed. In turn, our results suggest that both lineage specialists and generalists can show low chemical specialisation on intrageneric or intrafamilial host level, although the behavioural and physiological mechanisms can differ.

In contrast to beetles, both sawflies and caterpillars showed higher DSI* when accounting for host chemical β -diversity than when accounting for the simple number of hosts. Sawflies and caterpillars feeding on Salicaceae show contrasting host-plant preferences; sawflies that feed on Salicaceae are unlikely to feed on other families. On the other hand, many of the studied caterpillar species also feed on other plant families (Nyman et al., 2006; Volf, Hrcek, et al., 2015). Yet, both groups showed similar trends in their DSI* with respect to chemical β -diversity among the studied hosts. We originally expected that salicylates would play a stronger role in sawfly and caterpillar host-plant preference than flavonoids as they are a group of metabolites specific to Salicaceae that has been reported to retard larval growth, increase herbivore mortality or deter herbivores from feeding (Hjalten et al., 2007; Lehrman et al., 2012; Orians et al., 1997). Nevertheless,



specialisation relative to salicylates and flavonoids was not different from the one towards overall chemical β -diversity. Both metabolite groups are involved in host recognition and are used as feeding or oviposition cues by sawflies or female lepidopterans (Kolehmainen et al., 1994; Lahtinen et al., 2005; Roininen et al., 1999; Vihakas, 2014). Host flavonoids have been also reported to serve as antioxidants in insects or to be involved in cocoon formation, which can further increase their roles in sawfly and caterpillar specialisation (Kurioka & Yamazaki, 2002; Schopf et al., 1982).

Overall, our results suggest that β -diversity in salicylates probably did not restrain caterpillar and sawfly host spectra more strongly than β -diversity in flavonoids. We thus conclude that although salicylates are defences largely unique to the Salicaceae family, they do not have more pronounced effects on feeding preferences of multiple herbivore species associated with willows than flavonoids that are broadly distributed among plants. Together with the high preference of some specialists for salicylate-rich hosts (Ruuhola et al., 2001), such trends may have contributed to the loss of salicylates in some of the derived willow species, which seem to rely on other, more common, forms of defences (Volf, Julkunen-Tiitto, et al., 2015). It thus does not always have to be rare or species-specific metabolites that drive host-plant preference of leaf-chewing insects on intrageneric or intrafamilial level.

Qualitative differences in chemical β -diversity can affect insect herbivore performance and fitness (Roininen et al., 1999; Volf et al., 2018). In contrast, our results suggest that it was mainly the quantitative differences and not the presence or absence of metabolites that corresponded to changes in sawfly and caterpillar DSI*. The DSI* values accounting for qualitative chemical β -diversity did not differ from DSI* values not taking chemical composition of the hosts into account. Our results support Lehrman et al. (2012), who found a significant effect of salicylates on herbivores only when they accounted for quantitative changes in their total content. Although summing up multiple metabolites into a variable that does not take their exact structures into account does not always have to be informative (Volf et al., 2019), it can be useful when combining structurally related metabolites with similar activities. For example, total contents or diversities of hydrolysable tannins, alkaloids, or proteases can be informative on their activity against caterpillars and on herbivore community structure, specialisation, or diversity (Segar et al., 2017; Volf et al., 2018).

In conclusion, our results suggest that specialisation of insects related to willows and poplars may be linked primarily to quantitative changes in their chemistry rather than to tweaks leading to production of rare metabolites. Species from several speciose genera have been suggested to diverge in their defences in order to avoid sharing their insect herbivores (e.g. Kursar et al., 2009; Salazar et al., 2016; Volf et al., 2018). Our results suggest that plants may need to undergo quantitative changes in chemistry to significantly affect insect preferences, which in turn, could lead to sharing less herbivores. Changing the contents of major metabolite groups or switching to another form of defence completely can be thus an especially successful strategy how to avoid herbivory (Agrawal & Fishbein, 2006; Volf et al., 2018).

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Martin Volf and Leonardo R. Jorge designed the experimental approach; Leonardo R. Jorge conducted the statistical analysis. Jing Vir Leong wrote the first draft of the manuscript; all authors critically contributed to the final draft.

DATA AVAILABILITY STATEMENT

The data is openly available in the original studies of Volf et al. (2015) and are uploaded to the DRYAD repository.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Table S1. Chemical composition of the studied Salicaceae species including total number and average total content of salicylates and flavonoids (mg g⁻¹). Based on data from Volf et al. (2015b).

Table S2. List of studied host plants including number of plant individuals, total sampling effort time (min), total number of sampled herbivore individuals for each insect order, and total number of sampled herbivore species for each insect order found across all plant individuals for each respective host-plant species. Data from Volf et al. (2015).

Table S3. Model selection results explaining for variation in standardised distance-based specialisation index (DSI*). Comparison of different models of varying combinations and interactions of variables by accounting for insect order, β -diversity distance metric, and feeding preference with their respective corrected Akaike's information criterion scores (AICc), difference between the best model (dAICc), and their degrees of freedom (df).

Table S4. Pairwise comparisons based on linear mixed effect models for the differences between host specialisation and chemical β -diversity specialisation measurements (Binary - based on Jaccard's similarity index using presence/absence of all metabolite; Total - based on Bray-Curtis index using abundance of all metabolites; Flavonoids - based on Bray-Curtis index using flavonoid abundance; Salicylates - based on Bray-Curtis index using salicylate abundance; and Null - simple number of host plants disregarding their chemistry) within Coleoptera (a), Hymenoptera (b), and Lepidoptera (c). SE = standard error, df = degrees of freedom, bolded p-values indicate significance.

Table S5. Pairwise comparisons based on linear mixed effect models for the differences between insect orders with the following host specialisation and chemical β -diversity measurements: a) Binary - based on Jaccard's similarity index using presence/absence of all metabolites, b) Total - based on Bray-Curtis index using abundance of metabolites, c) Flavonoids - based on Bray-Curtis index using flavonoid abundance, d) Salicylates - based on Bray-Curtis index using salicylate abundance, and e) Null - simple number of host plants disregarding their chemistry. SE = standard error, df = degrees of freedom, bolded p-values indicate significance.

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