ECOLOGY LETTERS   cnrs WILEY

A mosaic of induced and non-induced branches promotes variation in leaf traits, predation and insect herbivore assemblages in canopy trees

Martin Volf[1,2] 🅾 | Tereza Volfová[1,3] 🅾 | Carlo L. Seifert[1,3,4] 🅾 | Antonia Ludwig[2,5] | Rolf A. Engelmann[2,5] | Leonardo Ré Jorge[1] 🅾 | Ronny Richter[2,5,6] 🅾 | Andreas Schedl[2,7] | Alexander Weinhold[2,7] 🅾 | Christian Wirth[2,5,8] 🅾 | Nicole M. van Dam[2,7] 🅾

[1]Institute of Entomology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic

[2]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

[3]Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

[4]Faculty of Forest Sciences and Forest Ecology, Department of Forest Nature Conservation, Georg-August-University, Göttingen, Germany

[5]Institute for Biology, University of Leipzig, Leipzig, Germany

[6]Geoinformatics and Remote Sensing, Institute for Geography, University of Leipzig, Leipzig, Germany

[7]Institute of Biodiversity, Friedrich Schiller University Jena, Jena, Germany

[8]Max-Planck Institute for Biogeochemistry, Jena, Germany

Correspondence
Martin Volf, Institute of Entomology, Biology Centre of the Czech Academy of Sciences, Branisovska 31, Ceske Budejovice, 37005, Czech Republic.
Email: volf@entu.cas.cz

Editor: Ted Turlings

Abstract

Forest canopies are complex and highly diverse environments. Their diversity is affected by pronounced gradients in abiotic and biotic conditions, including variation in leaf chemistry. We hypothesised that branch-localised defence induction and vertical stratification in mature oaks constitute sources of chemical variation that extend across trophic levels. To test this, we combined manipulation of plant defences, predation monitoring, food-choice trials with herbivores and sampling of herbivore assemblages. Both induction and vertical stratification affected branch chemistry, but the effect of induction was stronger. Induction increased predation in the canopy and reduced herbivory in bioassays. The effects of increased predation affected herbivore assemblages by decreasing their abundance, and indirectly, their richness. In turn, we show that there are multiple factors contributing to variation across canopies. Branch-localised induction, variation between tree individuals and predation may be the ones with particularly strong effects on diverse assemblages of insects in temperate forests.

KEYWORDS
canopy, diversity, herbivory, induced defences, polyphenols, predators, trophic interactions, vertical stratification, volatile organic compounds

INTRODUCTION

Forest canopies represent highly complex, three-dimensional environments (Lämke & Unsicker, 2018; Ulyshen, 2011). Abiotic and biotic conditions differ substantially among tree species, genotypes and even within individual trees, creating niches for various organisms (Bertić et al., 2021; Lämke & Unsicker, 2018; Seifert et al., 2020). Due to this variation, canopies harbour diverse communities of herbivorous insects and their natural enemies that form elaborate interaction webs (Gruner, 2004; Hunter et al., 1997; Valdés-Correcher, Moreira, et al., 2021). Studying how these trophic interactions are shaped by variation in leaf traits across the canopy can help to understand how the astonishing diversity of canopy organisms is maintained.

Various parts of the canopy are exposed to different abiotic conditions, forming a steep vertical stratification gradient from the forest floor to the uppermost canopy (Lämke & Unsicker, 2018). Increases in UV-irradiation, temperature or wind speed towards the upper canopy can drive variation in leaf toughness, phenolics or volatile organic compounds (VOCs) (Eisenring et al., 2021; Lämke & Unsicker, 2018; Murakami et al., 2005). Additional phenotypic variation among branches can also arise through localised somatic mutations or epigenetic factors (Padovan et al., 2013; Schmid-Siegert et al., 2017). The resulting differences in leaf quality and resource availability between lower and upper canopy layers drive interactions and species turnover in herbivore assemblages (Seifert et al., 2020; Ulyshen, 2011). Predation pressure changes along with these trends, typically increasing towards the upper canopy in response to its higher productivity and leaf turnover (Bridgeland et al., 2010; Van Bael et al., 2003). However, information on the exact drivers and consequences of the resulting changes in canopy trees is still remarkably scarce, especially for chemical leaf traits (Eisenring et al., 2021).

Recently, several studies suggested that herbivore-induced defences are an important source of variation in leaf quality and its effects on higher trophic levels in forest canopies (Lämke & Unsicker, 2018; Volf et al., 2020). After being attacked by herbivores, trees can upregulate direct defences, such as various phenolics, that are typical chemical defences of temperate broadleaf trees (Rossiter et al., 1988; Wold & Marquis, 1997). Herbivores show differential response to phenolics, ranging from reduced feeding to compensatory feeding, or even preference for such hosts (Damestoy et al., 2019; Roslin & Salminen, 2008; Segar et al., 2017). These contrasting effects can cause substantial turnover in herbivore assemblages between trees, depending on their level of phenolics (Segar et al., 2017).

Trees also employ indirect defences, such as various VOCs, that attract predators (McCormick et al., 2014; Volf et al., 2021). Herbivore-induced VOCs allow predators to navigate towards attacked plants, even in the absence of visual cues, and to immediately remove the herbivores (Amo et al., 2013). They are also readily produced, unlike direct chemical defences that may take longer to be synthesised at sufficient concentrations (Hunter, 1987; Volf et al., 2021). VOCs thus may play a prominent role in dynamic and complex ecosystems, such as canopies of temperate deciduous forests. However, most studies investigating the effects of VOCs focused on seedlings, relatively young trees or isolated individuals (e.g. Amo et al., 2013; McCormick et al., 2014; Volf et al., 2021). The roles of VOCs in trophic interactions in the complex canopies of natural forests are still widely unexplored (McCormick et al., 2019).

Induced defences can be highly localised within individual trees (Rubert-Nason et al., 2015; Volf et al., 2021). Herbivore-induced responses can be mediated by internal signalling or airborne signals. Their strength differs between the attacked site and distal tree parts, depending on their connection by the phloem, their spatial distance and the particular defensive trait (Heil & Ton, 2008). For example the activity of phenolics can be upregulated both systemically or locally in individual leaves (Wold & Marquis, 1997). Some VOCs, such as various terpenoids, seem to show especially localised upregulation in trees, being synthesised de novo in the induced foliage (McCormick et al., 2014; Volf et al., 2021). Such induced variation in tree chemistry can contribute to the constitutive variation due to vertical stratification or individual differences, resulting in a 'mosaic' of branches with different traits (Eisenring et al., 2021; Gripenberg et al., 2007; Padovan et al., 2013; Volf et al., 2021). The effects of such fine-scale variation can extend to higher trophic levels, thereby promoting variation in herbivore or predator assemblages associated with different parts of the canopy (Lämke & Unsicker, 2018; Volf et al., 2020).

Here we focus on the variation in leaf traits and trophic interactions across canopies of pedunculate oaks (*Quercus robur* L.) in order to expand our previous common garden experiments (Volf et al., 2021) into a natural setting and mature trees. Pedunculate oaks are key components of European floodplain forests (Richter et al., 2016). Oak defences, in particular phenolics and VOCs, have been extensively studied, showing that there is a high level of genetic and phenotypic variation among and within canopies of individual trees (Bertić et al., 2021; Roslin et al., 2006). This variation drives interactions with insect herbivores and their natural enemies, making some trees more susceptible to herbivory than others (Bertić et al., 2021). As in other temperate broadleaf trees, oak interactions are strongly seasonal, with the highest numbers of herbivores and their enemies occurring during the relatively short period of spring leaf flush (Le Corff et al., 2000; Volf et al., 2019). In our study, we focus on this particular part of the season as we expect variation in leaf chemistry to play the strongest role during this period when trees need to protect their young and valuable foliage (Bridgeland et al., 2010). Specifically, we focus on the roles of simulated

branch-localised defence induction in driving trophic interactions in the canopy, within the background of vertical stratification, and individual variation among oaks. Instead of describing individual links, we explore multiple interactions between oak traits, predation and herbivore assemblages in a diverse, natural forest. We specifically aim at connecting field studies on trophic interactions (e.g. Hunter et al., 1997; Le Corff et al., 2000) with experimental studies on canopy chemistry (e.g. McCormick et al., 2014; Eisenring et al., 2021).

We postulated that experimentally inducing leaf defences affects herbivore assemblages, either by changing herbivore preferences via increases in direct defences or by attracting more predators via enhanced VOC emissions. We expected that this effect would supersede patterns driven by the variation in leaf chemical traits due to intraspecific variation and vertical stratification. In turn, we show a complex network of correlations and interactions that drive the variation across canopies of mature oaks in a diverse floodplain forest. We demonstrate that not all these links are equal and identify the variation between tree individuals, vertical stratification and predation as those with the most pronounced effects on diverse assemblages of insect herbivores.

MATERIALS AND METHODS

Experimental site

We performed our experiments in April–May 2019 during the period of leaf flush using the Leipzig Canopy Crane facility (LCC) located within the floodplain forest of Leipzig (Saxony, Germany, 51.366 N, 12.309 E). The site is equipped with a 40-m-high Liebherr 71 EC crane on rails with a span covering 16,500 m^2. The crane plot includes nine mature pedunculate oaks reaching the upper canopy. In 2019, one individual was damaged by frost, leaving us with eight individuals that we used in our experiments (average diameter at breast height ~70 cm). We randomly selected 86 terminal branches across the eight trees that were at least 3 m apart (6–16 branches per tree, depending on the canopy size). The selected branches were ca 5-cm thick at their basis and were 18.0–32.8 m above ground. They carried 0.4–3.2 m^2 of foliage. We used half of the branches for the induction treatment and half as controls, evenly distributing the two categories along the vertical stratification gradient (Table 1). The studied branches were close to other foliage to allow wingless insects to move in response to our treatment.

Induction treatment

To induce defences in the treated branches, we used methyl jasmonate (MeJA), a derivative of jasmonic acid

TABLE 1 Oak individuals used in the experiment, including their ID number within the crane plot, diameter at breast height (DBH), total height and number of branches used in the experiment. Half of the branches were induced with methyl jasmonate, the other half was used as controls. The oaks showed an asynchronous leaf flush that affected the date when they entered our experiment and were treated with the induction treatment for the first time

Tree ID	DBH (cm)	Height (m)	Branches	1st induction treatment
086	29.8	23.7	6	May 2
129	134.0	43.9	16	April 25
278	103.3	38.2	12	May 9
317	80.5	32.4	12	May 9
397	69.0	34.4	12	May 9
449	35.0	28.9	8	April 30
733	52.0	29.5	10	April 25
751	46.8	26.1	10	April 25

involved in defence induction. MeJA is used in ecological studies to simulate herbivory by chewing-insects (Baldwin, 1996). While it does not provide the same level of specificity and intensity of induction as real herbivory, it allows for standardised comparisons between multiple trees and branches. We started treating individual trees once their leaves expanded and reached ca 2/3 of their final size. The leaf flush was asynchronous across our oak individuals. Therefore, we started treating individual trees on different dates (Table 1). This affected the timing of all other tasks described below.

We sprayed the branches in 3- to 4-day intervals with a MeJA (Sigma-Aldrich) aqueous solution with 1% EtOH and 0.1% Triton X-100 (Sigma-Aldrich) till run-off. We used 5 mM MeJA solution while the leaves were still expanding to avoid their necrosis. Once they reached their full size, we used 10 mM solution. Control branches were sprayed with the carrier solution. All trees were treated six times over a period of 2.5 weeks except for tree 449 that was treated seven times to synchronise its sampling with other trees. We used MeJA as a proxy for testing the effects of induced variation on leaf traits, herbivores and predation rates in oak canopies, which is why we used highly concentrated solutions to ensure a sufficiently strong plant response.

Predation monitoring

We used dummy caterpillars to estimate changes in predation rates across the canopy. We prepared the caterpillars (3 mm in diameter, 30 mm in length) using green non-toxic Newplast plasticine (Newclay Products). Using Loctite 401 super glue (Henkel), we attached three caterpillars to each branch immediately after each induction treatment (258 clay caterpillars placed in total). Each caterpillar was placed for 2.5 weeks, that is, for the

whole period when the given branch was subjected to the induction treatment. The caterpillars were checked for beak and bite marks in 3- to 4-day intervals and the damaged and missing ones were replaced.

Trait sampling

Following the last induction treatment on each tree, we sampled VOCs from all branches using polydimethylsiloxane (PDMS) tubes (Carl Roth GmbH) following Kallenbach et al. (2014). We placed two 1.5-cm PDMS tubes on a stainless-steel wire, attached it to the measured branch and enclosed it together with 19–170 leaves (depending on their size; we used leaf area to standardise VOC emissions) in a polyamide bag (45 × 55 cm; Studio Cook BV). The VOCs from the headspace were passively adsorbed onto the PDMS tubes that were collected after 24 h for logistical reasons. We observed some condensation in the bags. While this probably did not affect our ability to quantify terpenoids, it could affect the quantification of oxygenated VOCs that can dissolve in condensed water (Kallenbach et al., 2014). Placing the bags on the branches could also change the production of terpenoids due to the shortage of CO_2 (Affek & Yakir, 2003). Such changes would occur across all branches and thus would have limited effects on our conclusions.

Additionally, we estimated the number of leaves on each branch and haphazardly sampled 4–7 of them once we completed all induction treatments of the given branch. We photographed the leaves and analysed their area in ImageJ (Abràmoff et al., 2004). We calculated the leaf area of each branch by extrapolating the area of photographed leaves by the estimated total number of leaves on the given branch. We used three fresh leaves per branch to measure leaf toughness using FL50 penetrometer with Stepper Motor Powered Test Stand TVO 500N55S (Sauter GmbH) and a 1.5-mm-wide tip. Each leaf was measured three times avoiding the main veins and the values were averaged. Finally, three leaves per branch were freeze-dried, homogenised and used for FT-IR analysis of polyphenols. The remaining leaf material was used for food-choice trials with caterpillars.

Food-choice trials

We compared the palatability of leaves from control and treated branches to polyphagous *Lymantria dispar* (Linnaeus, 1758) caterpillars in food-choice trials. Individual freshly hatched caterpillars were placed in round plastic arenas with two 1.5-cm leaf discs. Each trial involved one disc from a treated branch and a disc from the closest control branch from the same tree and a similar height above ground. The caterpillars fed for 24 h. Thereafter, the leaf discs were photographed and the remaining leaf area was measured in ImageJ (Abràmoff

et al., 2004). We ran up to three trials per branch pair. Caterpillars that did not feed were removed from the dataset, resulting in 100 trials we analysed.

Insect sampling

Three days after the last induction treatment (ca. three weeks after the first induction treatment), we comprehensively sampled leaf-chewing caterpillars and sawfly larvae from all branches by a combination of beating and manual searching following Volf et al. (2019). Due to the asynchronous leaf flush, the first trees were sampled on May 17, while the last one was sampled on May 30. Larvae were morphotyped, photographed, and reared to adults (Volf et al., 2019). Dead larvae were preserved in ethanol. Final identifications were based on larval and adult morphology. Additionally, 190 insect individuals were barcoded at CCDB. COI barcode-sequences (658 bp) were uploaded to the Barcode of Life Database (www.bolds ystems.org, dx.doi.org/10.5883/DS-LAK2019) and the Barcode Index Numbers were used to improve our species identifications (Ratnasingham & Hebert, 2013). The insect abundance rapidly decreased in the months following our sampling, which is a common seasonal trend in temperate forests (Le Corff et al., 2000; Volf et al., 2019). Therefore, we did not repeat the sampling later in the season as we would not be able to get representative insect samples from the studied branches. This limits the conclusions we can draw on delayed effects of induced defences on trophic interactions.

Chemical analysis

The VOCs adsorbed to PDMS cuttings were analysed by thermal desorption-gas chromatograph-mass spectrometry (TD-GC-MS) following the methods described in Volf et al. (2021); see Appendix S1 for details. Detected VOCs were tentatively identified by comparisons to the NIST20 database (National Institute of Standards and Technology, Gaithersburg, MD, USA) and comparisons to retention indices from the literature. The peak areas of individual VOCs were calculated using the Bruker Workstation software (v8.0.1) and used in the subsequent analyses.

We quantified the concentration of total phenolics and hydrolysable tannins (HHDPs; hexahydroxydiphenic acid derivatives) in the leaf samples using an FT-NIR analysis. To perform spectral measurements, we used 60–65 mg of ground and freeze-dried leaf samples. To predict the concentration of total phenolics and HHDPs in our samples based on the spectral measurements, we created a predictive model that we calibrated using 41 leaf samples collected during our previous projects (Volf et al., 2021). We used a modified version of the CARS-PLS framework as described in Elle et al. (2019)

to relate the measured spectra of the 41 samples to the measured total phenolics and HHDPs in these samples. To finally determine the total phenolics and HHDPs in a specific sample and to estimate prediction accuracies for samples unseen in the model, predictions from the ensemble model were averaged using the median concentration (Appendix S1).

Statistical analysis

We explored the causal relationships between the induction, vertical stratification, studied oak traits and higher trophic levels with *Structural Equation Modelling* (SEM). SEM allows to test for direct effects between pairs of variables and for indirect effects of other variables involved in the network. We used ten variables describing the induction treatment, vertical stratification, VOC emission level, VOC composition, HHDPs, leaf toughness, predation, herbivore preference, herbivore abundance and herbivore richness (see Table 2 and Table S1 for details). These variables were used to build a system of structural equations, employing local estimation methods, with the biologically plausible causal relationships included *a priori* in the model (Figure S1). Given the blocked design of the experiment within trees, all the relationships were modelled employing mixed models, with tree as a random factor. For herbivore richness, we employed a generalised linear mixed model, with a Poisson distribution and a log link function, while all other models were linear mixed models. We used the package piecewiseSEM (Lefcheck, 2016) to fit the model. We evaluated the quality of the fit of our reduced model when compared to a saturated model, by making a direct separation test

using Fisher's C statistic. We used the marginal and conditional R^2 values for each of the response variables to assess the explanatory power of our model and disentangle the effect of individual trees and explanatory variables.

Then, we explored the trends in more detail in the case of variables for which we had more individual components characterising them. First, we performed partial *Redundancy Analysis* (RDA) to test for the detailed effects of the induction treatment and stratification on VOC composition. We used the treatment and vertical stratification as explanatory variables and the tree individual as a covariable defining the permutation blocks. We log-transformed the individual VOCs, used them as response variables, tested the significance of all canonical axes using 9999 permutations, and adjusted the explained variability following ter Braak and Smilauer (2012). Second, we ran two separate *Linear Mixed Effect Models* (LMEs) testing the response in HHDPs (an anti-herbivore defence) and total polyphenol content (accounting for various polyphenols with various functions) to see if there was any difference in their correlation to the treatment and vertical stratification. We used the treatment and vertical stratification as fixed effects, the tree individual as a random factor and compared log-transformed HHDP content and total phenolics across the studied branches in R 3.6.1 (R Core Team, 2019) using 'lme4' package (Bates et al., 2015). Third, we tested the effect of individual VOCs on predation rates. We employed LMEs, with the predation rate as the response variable, tree individual as a random factor and each of the VOCs as the fixed effect variable. We built 49 separate models, one for each VOC, and accounted for the multiple comparisons following Hommel (1988). Finally, we tested the effects of induction treatment and

TABLE 2 Variables used in structural equation modelling. All variables were measured on a branch-basis. If there were multiple values for a branch, we used their average

| Variable	Description
Induction treatment	Induction treatment with methyl jasmonate; coded as 1 (for treatment) and 0 (for control)
Vertical stratification	Height above ground (in m)
VOC emission level	Total area under the peaks of individual VOCs we measured with GC-MS
VOC composition	Similarity in VOC profiles emitted by the studied branches as measured on the first PCA axis; based on log-transformed emission levels of VOCs as analysed by PCA in Canoco 5 (ter Braak et al., 2012)
Leaf toughness	Leaf toughness (in N); an average value based on three leaves per branch
HHDPs	Content of HHDPs (in mg/g) based on FT-IR
Predation	Predation rates (0.00–1.00) at the given branch as estimated with dummy caterpillars. The rates were calculated as the proportion between the number of recorded predation events and the number of caterpillars checked on the given branch
Herbivore preference	Average area of leaf discs eaten by *Lymantria dispar* in food-choice trials (in mm). Each trial involved one disc from a treated branch and a disc from the closest control branch from a similar height above ground. The comparisons were replicated up to three times and the average value was used
Herbivore abundance	Number of caterpillars and sawfly larvae sampled from the given branch standardised by its leaf area; we applied sqrt transformation to the data
Herbivore richness	Species richness of caterpillars and sawfly larvae sampled from the given branch

vertical stratification on the herbivore assemblages with *Canonical Correspondence Analysis* (CCA). We used the induction treatment and vertical stratification as explanatory variables and the tree individual as a covariable defining the permutation blocks. We down-weighted rare species, tested the significance of all canonical axes using 9999 permutations, and adjusted the explained variability following ter Braak and Smilauer (2012). We removed species occurring only on one branch from all multivariate analyses.

RESULTS

The SEM analysis yielded a good representation of the overall correlation structure of the variables studied, with no significant deviation from goodness-of-fit (Fisher's C = 28.76, d.f. = 28, $p = 0.425$). The significant correlations obtained in the SEM are shown in Figure 1, along with the marginal and conditional R^2 values for all the response variables.

Leaf toughness and HHDPs showed a stronger positive correlation with the induction treatment than with vertical stratification, with the effect of stratification on HHDPs not being significant (Figure 1, Table S2). Both traits were also strongly influenced by the variation among oak individuals (Table S3). The effect of induction on HHDPs was confirmed by LMEs comparing them to total phenolics. HHDP content was positively correlated with the induction treatment (χ^2 (1) = 12.48, $p < 0.001$) but it showed no significant correlation to vertical stratification (χ^2 (1) = 0.01, $p = 0.906$). Contrastingly, the total phenolics showed no significant correlation to the induction treatment (χ^2 (1) = 0.41, $p = 0.520$), while they increased towards the upper canopy (χ^2 (1) = 15.21, $p < 0.001$; Table S4).

In total, we measured 49 VOCs, mainly mono- and sesquiterpenes or derivatives thereof (Table S5). Out of these, 11 were missing from the control branches, whereas all were present in at least five induced branches. VOC emission level and composition showed a strong correlation to the induction treatment (Figure 1, Table S2). Both
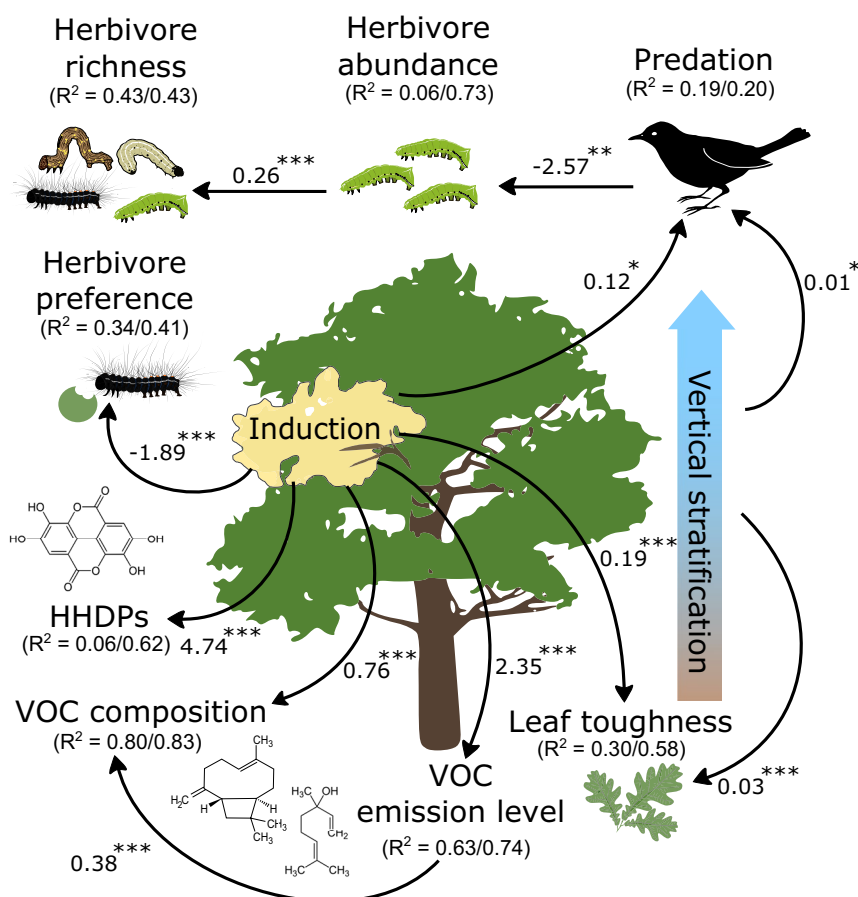
FIGURE 1 The causal relationships between the induction treatment, vertical stratification, plant traits, predation and herbivores in oak canopies. The arrows show significant relationships as analysed by structural equation modelling. The values above arrows show model estimates and asterisks indicate significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; Table S2). Values below the response variable labels show marginal R^2 (representing the variance explained by the fixed effects) and the conditional R^2 (representing the variance explained by both the fixed and random effects [i.e. by the oak individual]). All models were fit with linear mixed effects models, except for herbivore richness, which used a generalised linear mixed model with Poisson error distribution and a log link function. Only the significant relationships are shown (see Figure S1 for all the relationships tested)

VOC emission level and composition were only weakly affected by differences between oak individuals (Table S3). This was also confirmed by the RDA that showed most of the VOCs, in particular mono- and sesquiterpenes, to be strongly upregulated in the treated branches (Figure 2). The induction treatment explained 25.3% of the adjusted variability in the VOCs (pseudo-F = 27.5, $p < 0.001$). The effect of vertical stratification was not significant (pseudo-F = 1.2, $p = 0.265$, 0.25% of the adjusted variability explained).

Caterpillars preferred to feed on discs from control branches over those from treated branches, irrespective of vertical stratification, HHDPs, or leaf toughness (Figure 1, Table S3). In the field, we sampled 898 caterpillars and sawfly larvae representing 41 species (Table S6). Neither their abundance nor their richness was directly correlated with vertical stratification, treatment or oak traits. Herbivore abundance varied greatly among oak individuals (Table S3). Similar to the results of the path analysis, the results of CCA showed that the induction treatment had no effect on the herbivore assemblages (pseudo-F = 0.6, $p = 0.911$, 0.0% of the adjusted variability explained). Although the herbivore assemblage composition was significantly correlated with stratification (pseudo-F = 1.5, $p = 0.048$), the adjusted explained variability was only 0.6% (Figure 3). The variability explained by oak individual was 10.9%.

We recorded 278 predation events. Predation rates were positively correlated with both vertical stratification and induction treatment, whereas the effect of oak individual was relatively small (Table S2). The predation rates were not correlated with overall VOC emission level or composition (Figure 1). We found five individual terpenoids, caryophyllene, (*E*)-4,8-dimethylnona-1,3,7-triene, hydroxy-eucalyptol, linalool and trans-linalool oxide, whose emissions correlated positively to the predation rates. However, these correlations disappeared when we applied a multiple-comparison correction (Table S7). The effects of induction and vertical stratification on predation extended to herbivores, whereby predation had a negative effect on herbivore abundance, and eventually herbivore richness (Figure 1).

DISCUSSION

Diverse canopy communities are maintained by pronounced gradients in abiotic and biotic factors (Hunter et al., 1997; Lämke & Unsicker, 2018; Ulyshen, 2011). We studied how the effects of branch-localised defence induction, vertical stratification and differences between oak individuals cause variation in leaf traits that extends to higher trophic levels and diverse insect assemblages. We show that branch-localised induction
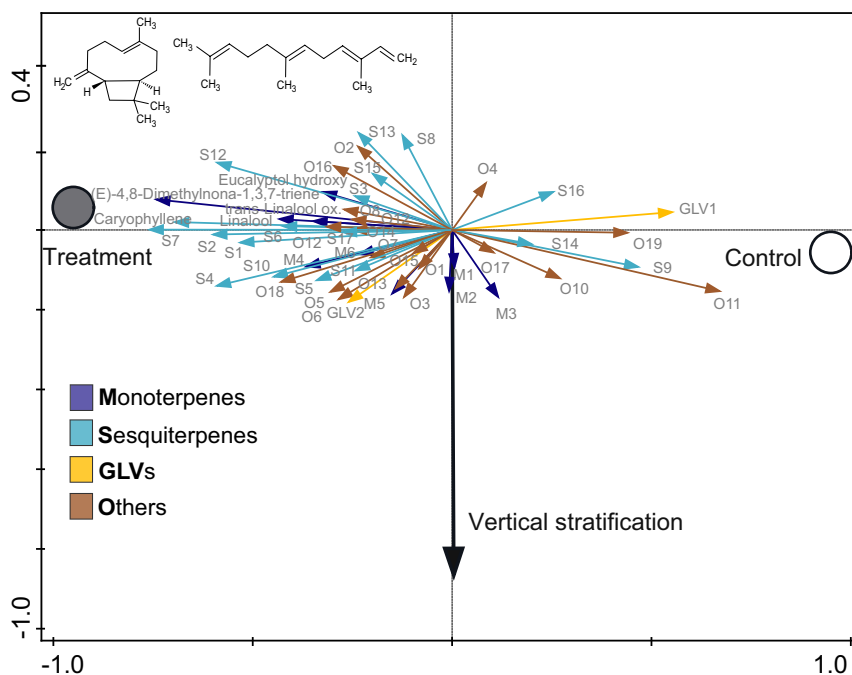
FIGURE 2 Correlation between the VOC profiles, induction treatment and vertical stratification as analysed by a partial RDA with 9999 permutations. Most of the VOCs were strongly upregulated in the treated branches, although some were also downregulated in response to the treatment. The treatment explained 25.3% of the adjusted variability in VOC profiles (pseudo-F = 27.5, $p < 0.001$). The effect of vertical stratification was not significant (pseudo-F = 1.2, $p = 0.2650$, 0.25% of the adjusted variability explained). VOCs are shown as coloured arrows (Monoterpenes in light blue, Sesquiterpenes in dark blue, Green Leaf Volatiles (GLVs) in orange, and Other and unidentified compounds in brown). Vertical stratification (height above ground; a continuous explanatory variable) is shown as a thick black arrow and the treatment (as a two-level categorical explanatory variable) is shown as circles
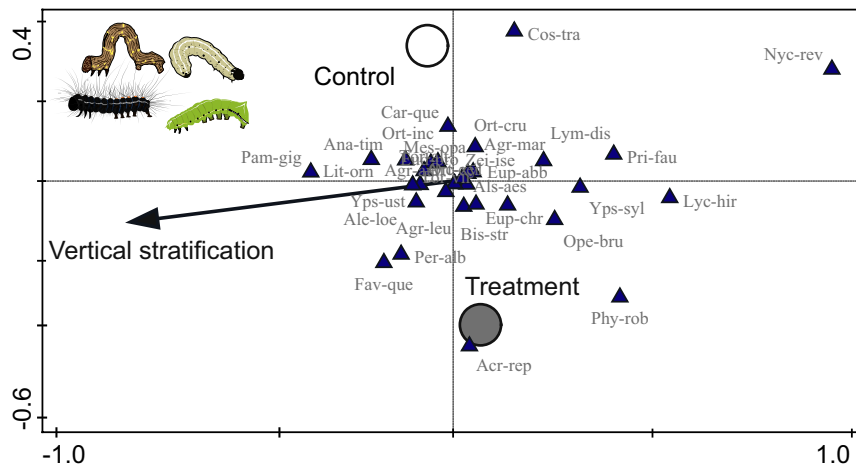
FIGURE 3 Correlation of the caterpillar assemblage composition with the induction treatment and vertical stratification as analysed by a partial CCA with 9999 permutations. The treatment had no effect on the studied assemblages (pseudo-F = 0.6, p = 0.9111, 0.0% of the adjusted variability explained). In contrast, the community composition was significantly correlated with stratification (pseudo-F = 1.5, p = 0.0475), although the adjusted explained variation was small (0.6%). Triangles represent insect species, vertical stratification (height above ground; a continuous explanatory variable) is shown as an arrow and the treatment (as a two-level categorical explanatory variable) is shown as circles. See Table S6 for insect species codes

increases predation risk, which reduces herbivore abundance in highly dynamic oak canopies during leaf flush. Contrastingly, herbivore assemblages were not affected by any leaf trait we measured, despite their pronounced induction. Additionally, the identity of oak individual and vertical stratification affected herbivore assemblages to different extents.

The induction strongly promoted variation in oak traits that are primarily involved in trophic interactions with herbivores and predators. The effect of induction was particularly strong in the case of VOCs, with some terpenoids occurring exclusively in the induced branches. In contrast, we did not find any significant increase in VOCs towards the upper canopy of mature trees, although VOCs can increase in young oaks in response to UV-irradiation (Harley et al., 1996) and aid plant performance under abiotic stress (Frank et al., 2021). Our results support studies in trees that suggested a high importance of localised upregulation of terpenoids, which are synthesised de novo in the induced tissue (McCormick et al., 2019; Volf et al., 2021). The largely localised induction probably reflects large spatial distances and differences in connectivity between individual branches, which can limit signalling via phloem. This suggests that the induction stimuli probably need to be relatively strong to cause a systemic response via phloem signalling in mature trees (Heil & Ton, 2008; McCormick et al., 2019). The efficiency of airborne signals strongly depends on the abiotic conditions and the metabolites in play (Douma et al., 2019), which can further contribute to the compartmentalisation of defences across canopies. From the defence-economics perspective, such localised induction may allow large trees to fine-tune their defences to match the variation in herbivory pressure over the canopy. In case of VOCs,

branch-localised induction could theoretically improve their communication with predators and parasitoids by attracting them specifically to the damaged part of the canopy (Lämke & Unsicker, 2018; McCormick et al., 2019; Volf et al., 2021).

Various terpenoids, and particularly those synthesised de novo in response to the induction, can be reliable cues attracting predators to damaged trees (Amo et al., 2013; McCormick et al., 2012). Five individual VOCs, all terpenoids, significantly correlated with predation rates in our experiment, although this correlation disappeared after correcting for multiple comparisons. Interestingly, two of these VOCs, linalool and (E)-4,8-dimethylnona-1,3,7-triene ((E)-DMNT), are associated with caterpillar damage and predator attraction in other tree species (Mäntylä, Alessio, et al., 2008; McCormick et al., 2019). These studies used real caterpillars to induce the trees and yet obtained similar results as our study using MeJA-based induction, which supports the notion that enhanced terpenoid emissions in trees can enhance predation in forest canopies. However, the absence of a clear correlation between overall VOC emission level and composition also suggests that there could be additional factors contributing to the increase in predation rates on the induced branches. For example our induction treatment could change leaf reflectance so they would resemble leaves attacked by herbivores and attract predators (Mäntylä, Klemola, et al., 2008). Both vision and VOCs thus likely help predators navigate towards the induced branches through the dense canopy foliage.

The effect of predation was quite stable across the trees, possibly due to high predator mobility. Both vertebrate and invertebrate predators have been shown to reduce herbivory in trees (Böhm et al., 2011; Van Bael et al., 2003). Predation is generally higher at low latitudes

and elevations, in forests with higher leaf production, or on individual fast-growing trees (Bridgeland et al., 2010; Roslin et al., 2017; Van Bael & Brawn, 2005). Predation also typically increases towards the productive sun-exposed canopy (Van Bael et al., 2003) as evidenced by our study. Additionally, our results suggest that predators play a strong role in the dynamic canopies of temperate forests during leaf flush. The role of predators thus probably increases with productivity independent of the scale one looks at within forest ecosystems (Borer et al., 2006; Fretwell & Barach, 1977). Here, the predators could readily remove herbivores from the branches, providing an effective protection for young, nutrient-rich leaves. By lowering their abundance, predators indirectly affected herbivore diversity, creating a mosaic of branches harbouring different herbivores.

Similarly, we also found a branch-localised increase in HHDPs and leaf toughness. Both localised and systemic induction of direct defences occur in oaks in response to herbivory (Rossiter et al., 1988; Wold & Marquis, 1997). Early instars of many generalist, spring-feeding insect larvae are able to escape from non-suitable trees or branches (Hunter, 1990; Ramachandran, 1987). However, we did not record any such effect in the canopy, and we observed a negative effect of the induction on insect larvae only in the laboratory. Instead, the composition of insect assemblages in the canopy was primarily affected by intraspecific variation between oak individuals. Pedunculate oaks are highly variable in their chemistry, forming ecotypes with differential resistance to herbivores, as also suggested by the high intraspecific variation in both HHDPs and leaf toughness in our system (Bertić et al., 2021; Ghirardo et al., 2012; Roslin et al., 2006). Furthermore, oaks show pronounced differences in the timing of their leaf flush. Foliage availability is a key factor structuring assemblages of spring insect herbivores in temperate forests (Hunter, 1990, 1992). Much of the observed variation in herbivore assemblages could thus be attributed to differences in bud burst and foliage availability among the studied oaks. This is further supported by the fact that we observed a modest correlation between herbivore assemblages and vertical stratification, which has been previously related to differences in foliage availability between canopy strata in a similar system (Seifert et al., 2020).

The vertical stratification was also associated with an increase in leaf toughness and total phenolics. Both these traits allow leaves in the upper canopy to withstand high intensities of UV-irradiation and wind, while maximising their photosynthetic capacity under high light availability (Eisenring et al., 2021; Murakami et al., 2005; Valdés-Correcher, Bourdin, et al., 2021). Their increase thus can be unrelated to the variation in biotic interactions across the canopy and result mainly from the role they serve in protecting leaves against abiotic factors. While crown position is one of the main sources of variation in chemical traits across the canopy (Eisenring et al., 2021), the specific trends in this variation may depend on the exact drivers and roles the individual traits play in tree defence and physiology.

In conclusion, we show that forest canopies are highly dynamic systems where induced responses, intraspecific variation and vertical stratification play important roles (Eisenring et al., 2021; Lämke & Unsicker, 2018). Together with molecular mechanisms such as large number of somatic mutations and epigenetic variation among distant branches, these mechanisms contribute to high variation of chemical traits across the canopy (Lämke & Unsicker, 2018; McCormick et al., 2019; Padovan et al., 2013; Schmid-Siegert et al., 2017; Volf et al., 2021). In turn, leaf traits and biotic interactions can be less predictable between individual branches than between individual oak trees in some cases (Gripenberg et al., 2007). However, the contributions of induced responses, intraspecific variation and vertical stratification are not equal. While previous studies explored pairwise interactions in young or isolated trees, their chemistry and higher trophic levels, we identified effects with major roles in natural forest canopies. Based on our results, intraspecific variation between tree individuals and predation have particularly strong effects on herbivore abundance and richness during leaf flush. In contrast, upregulation of direct defences may primarily affect following generations of herbivores and structure their assemblages on longer time scales (Boeckler et al., 2013; Hunter, 1987). Various herbivores may also show differential responses to top-down and bottom-up effects (Hunter et al., 1997). This can create important interactions between various chemical defences in oaks in terms of providing efficient defence against herbivory. Although difficult to detect, such interactions between plant traits, predation and various abiotic factors are probably key factors that promote spatial and temporal variation within and among tree canopies and contribute to their high diversity.

AUTHORSHIP

MV, TV, CLS, AW, CW and NMvD designed the experimental approach; MV, TV, CLS, AL, RAE and RR collected the data; MV, RR, AS and AW conducted the chemical analysis; MV, LRJ and RR conducted the statistical analyses; MV wrote the first draft of the manuscript; all authors critically contributed to the final draft.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ele.13943.

DATA AVAILABILITY STATEMENT

The data on measured branch variables, VOCs from individual branches and total numbers of larvae collected from treated and control branches in individual trees are available on Zenodo (https://zenodo.org, dx.doi.org/10.5281/zenodo.5705300). The data on COI sequences used for insect identification including the standard fields for the BARCODE data standard are available on BOLD as LAK project (www.boldsystems.org, dx.doi.org/10.5883/DS-LAK2019).

ORCID

Martin Volf https://orcid.org/0000-0003-4126-3897
Tereza Volfová https://orcid.org/0000-0001-8706-6819
Carlo L. Seifert https://orcid.org/0000-0002-2295-2833
Leonardo Ré Jorge https://orcid.org/0000-0003-4518-4328
Ronny Richter https://orcid.org/0000-0002-8728-7918
Alexander Weinhold https://orcid.org/0000-0003-1418-7788
Christian Wirth https://orcid.org/0000-0003-2604-8056
Nicole M. van Dam https://orcid.org/0000-0003-2622-5446

REFERENCES

Abràmoff, M.D., Magalhães, P.J. & Ram, S.J. (2004) Image processing with ImageJ. *Biophotonics International*, 11, 36–42.

Affek, H.P. & Yakir, D. (2003) Natural abundance carbon isotope composition of isoprene reflects incomplete coupling between isoprene synthesis and photosynthetic carbon flow. *Plant Physiology*, 131, 1727–1736.

Amo, L., Jansen, J.J., van Dam, N.M., Dicke, M. & Visser, M.E. (2013) Birds exploit herbivore-induced plant volatiles to locate herbivorous prey. *Ecology Letters*, 16, 1348–1355.

Baldwin, I.T. (1996) Methyl jasmonate-induced nicotine production in Nicotiana attenuata: inducing defenses in the field without wounding. In: Städler, E., Rowell-Rahier, M. & Baur, R. (Eds.) *Proceedings of the 9th International Symposium on Insect-Plant Relationships*. Springer, pp. 213–220.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Bertić, M., Schroeder, H., Kersten, B., Fladung, M., Orgel, F., Buegger, F. et al. (2021) European oak chemical diversity–from ecotypes to herbivore resistance. *New Phytologist*, 232, 818–834.

Boeckler, G.A., Gershenzon, J. & Unsicker, S.B. (2013) Gypsy moth caterpillar feeding has only a marginal impact on phenolic compounds in old-growth black poplar. *Journal of Chemical Ecology*, 39, 1301–1312.

Böhm, S.M., Wells, K. & Kalko, E.K. (2011) Top-down control of herbivory by birds and bats in the canopy of temperate broad-leaved oaks (*Quercus robur*). *PLoS One*, 6, e17857.

Borer, E.T., Halpern, B.S. & Seabloom, E.W. (2006) Asymmetry in community regulation: effects of predators and productivity. *Ecology*, 87, 2813–2820.

Bridgeland, W.T., Beier, P., Kolb, T. & Whitham, T.G. (2010) A conditional trophic cascade: birds benefit faster growing trees with strong links between predators and plants. *Ecology*, 91, 73–84.

Clavijo mccormick, A., Irmisch, S., Reinecke, A., Boeckler, G.A., Veit, D., Reichelt, M. et al. (2014) Herbivore-induced volatile emission in black poplar: regulation and role in attracting herbivore enemies. *Plant, Cell & Environment*, 37, 1909–1923.

Damestoy, T., Brachi, B., Moreira, X., Jactel, H., Plomion, C. & Castagneyrol, B. (2019) Oak genotype and phenolic compounds differently affect the performance of two insect herbivores with contrasting diet breadth. *Tree Physiology*, 39, 615–627.

Douma, J.C., Ganzeveld, L.N., Unsicker, S.B., Boeckler, G.A. & Dicke, M. (2019) What makes a volatile organic compound a reliable indicator of insect herbivory? *Plant, Cell & Environment*, 42, 3308–3325.

Eisenring, M., Unsicker, S.B. & Lindroth, R.L. (2021) Spatial, genetic and biotic factors shape within-crown leaf trait variation and herbivore performance in a foundation tree species. *Functional Ecology*, 35, 54–66.

Elle, O., Richter, R., Vohland, M. & Weigelt, A. (2019) Fine root lignin content is well predictable with near-infrared spectroscopy. *Scientific Reports*, 9, 1–11.

Frank, L., Wenig, M., Ghirardo, A., Krol, A., Vlot, A.C., Schnitzler, J.-P. et al. (2021) Isoprene and β-caryophyllene confer plant resistance via different plant internal signalling pathways. *Plant, Cell & Environment*, 44, 1151–1164.

Fretwell, S.D. & Barach, A.L. (1977) The regulation of plant communities by the food chains exploiting them. *Perspectives in Biology and Medicine*, 20, 169–185.

Ghirardo, A., Heller, W., Fladung, M. & Schnitzler, J.P. & Schroeder, H. (2012) Function of defensive volatiles in pedunculate oak (*Quercus robur*) is tricked by the moth *Tortrix viridana*. *Plant, Cell & Environment*, 35, 2192–2207.

Gripenberg, S., Salminen, J.P. & Roslin, T. (2007) A tree in the eyes of a moth–temporal variation in oak leaf quality and leaf-miner performance. *Oikos*, 116, 592–600.

Gruner, D.S. (2004) Attenuation of top-down and bottom-up forces in a complex terrestrial community. *Ecology*, 85, 3010–3022.

Harley, P., Deem, G., Flint, S. & Caldwell, M. (1996) Effects of growth under elevated UV-B on photosynthesis and isoprene emission in *Quercus gambelii* and *Mucuna pruriens*. *Global Change Biology*, 2, 149–154.

Heil, M. & Ton, J. (2008) Long-distance signalling in plant defence. *Trends in Plant Science*, 13, 264–272.

Hommel, G. (1988) A stagewise rejective multiple test procedure based on a modified Bonferroni test. *Biometrika*, 75, 383–386.

Hunter, M.D. (1987) Opposing effects of spring defoliation on late season oak caterpillars. *Ecological Entomology*, 12, 373–382.

Hunter, M.D. (1990) Differential susceptibility to variable plant phenology and its role in competition between two insect herbivores on oak. *Ecological Entomology*, 15, 401–408.

Hunter, M.D. (1992) A variable insect–plant interaction: the relationship between tree budburst phenology and population levels of insect herbivores among trees. *Ecological Entomology*, 17, 91–95.

Hunter, M.D., Varley, G.C. & Gradwell, G.R. (1997) Estimating the relative roles of top-down and bottom-up forces on insect herbivore populations: a classic study revisited. *Proceedings of the National Academy of Sciences*, 94, 9176–9181.

Kallenbach, M., Oh, Y., Eilers, E.J., Veit, D., Baldwin, I.T. & Schuman, M.C. (2014) A robust, simple, high-throughput technique for time-resolved plant volatile analysis in field experiments. *The Plant Journal*, 78, 1060–1072.

Lämke, J.S. & Unsicker, S.B. (2018) Phytochemical variation in tree-tops: causes and consequences for tree-insect herbivore interactions. *Oecologia*, 187, 377–388.

Le Corff, J., Marquis, R. & Whitfield, J. (2000) Temporal and spatial variation in a parasitoid community associated with the herbivores that feed on Missouri *Quercus*. *Environmental Entomology*, 29, 181–194.

Lefcheck, J.S. (2016) piecewiseSEM: piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, 7, 573–579.

Mäntylä, E., Alessio, G.A., Blande, J.D., Heijari, J., Holopainen, J.K., Laaksonen, T. et al. (2008) From plants to birds: higher avian predation rates in trees responding to insect herbivory. *PLoS One*, 3, e2832.

Mäntylä, E., Klemola, T., Sirkiä, P. & Laaksonen, T. (2008) Low light reflectance may explain the attraction of birds to defoliated trees. *Behavioral Ecology*, 19, 325–330.

McCormick, A.C., Irmisch, S., Boeckler, G.A., Gershenzon, J., Köllner, T.G. & Unsicker, S.B. (2019) Herbivore-induced volatile emission from old-growth black poplar trees under field conditions. *Scientific Reports*, 9, 1–10.

McCormick, A.C., Unsicker, S.B. & Gershenzon, J. (2012) The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. *Trends in Plant Science*, 17, 303–310.

Murakami, M., Yoshida, K., Hara, H. & Toda, M.J. (2005) Spatio-temporal variation in Lepidopteran larval assemblages associated with oak, *Quercus crispula*: the importance of leaf quality. *Ecological Entomology*, 30, 521–531.

Padovan, A., Keszei, A., Foley, W.J. & Külheim, C. (2013) Differences in gene expression within a striking phenotypic mosaic *Eucalyptus* tree that varies in susceptibility to herbivory. *BMC Plant Biology*, 13, 1–12.

R Core Team (2019) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.R-project.org Vienna, Austria

Ramachandran, R. (1987) Influence of host-plants on the wind dispersal and the survival of an Australian geometrid caterpillar. *Entomologia Experimentalis Et Applicata*, 44, 289–294.

Ratnasingham, S. & Hebert, P.D. (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS One*, 8, e66213.

Richter, R., Reu, B., Wirth, C., Doktor, D. & Vohland, M. (2016) The use of airborne hyperspectral data for tree species classification in a species-rich Central European forest area. *International Journal of Applied Earth Observation and Geoinformation*, 52, 464–474.

Roslin, T., Gripenberg, S., Salminen, J.-P., Karonen, M., B. O'Hara, R., Pihlaja, K. et al. (2006) Seeing the trees for the leaves–oaks as mosaics for a host-specific moth. *Oikos*, 113, 106–120.

Roslin, T., Hardwick, B., Novotny, V., Petry, W.K., Andrew, N.R., Asmus, A. et al. (2017) Higher predation risk for insect prey at low latitudes and elevations. *Science*, 356, 742–744.

Roslin, T. & Salminen, J.P. (2008) Specialization pays off: contrasting effects of two types of tannins on oak specialist and generalist moth species. *Oikos*, 117, 1560–1568.

Rossiter, M., Schultz, J.C. & Baldwin, I.T. (1988) Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. *Ecology*, 69, 267–277.

Rubert-Nason, K.F., Couture, J.J., Major, I.T., Constabel, C.P. & Lindroth, R.L. (2015) Influence of genotype, environment, and gypsy moth herbivory on local and systemic chemical defenses in trembling aspen (*Populus tremuloides*). *Journal of Chemical Ecology*, 41, 651–661.

Schmid-Siegert, E., Sarkar, N., Iseli, C., Calderon, S., Gouhier-Darimont, C., Chrast, J. et al. (2017) Low number of fixed somatic mutations in a long-lived oak tree. *Nature Plants*, 3, 926–929.

Segar, S.T., Volf, M., Isua, B., Sisol, M., Redmond, C.M., Rosati, M.E. et al. (2017) Variably hungry caterpillars: predictive models and foliar chemistry suggest how to eat a rainforest. *Proceedings of the Royal Society of London B: Biological Sciences*, 284, 20171803.

Seifert, C.L., Lamarre, G.P.A., Volf, M., Jorge, L.R., Miller, S.E., Wagner, D.L. et al. (2020) Vertical stratification of a temperate forest caterpillar community in eastern North America. *Oecologia*, 192, 501–514.

ter Braak, C. & Smilauer, P. (2012) Canoco 5, Windows release (5.00). Software for mutivariate data exploration, testing, and summarization. Biometris, Plant Research International, Wageningen.

Ulyshen, M.D. (2011) Arthropod vertical stratification in temperate deciduous forests: implications for conservation-oriented management. *Forest Ecology and Management*, 261, 1479–1489.

Valdés-Correcher, E., Bourdin, A., González-Martínez, S.C., Moreira, X., Galmán, A., Castagneyrol, B. et al. (2021) Leaf chemical defences and insect herbivory in oak: accounting for canopy position unravels marked genetic relatedness effects. *Annals of Botany*, 126, 865–872.

Valdés-Correcher, E., Moreira, X., Augusto, L., Barbaro, L., Bouget, C., Bouriaud, O. et al. (2021) Search for top-down and bottom-up drivers of latitudinal trends in insect herbivory in oak trees in Europe. *Global Ecology and Biogeography*, 30, 651–665.

Van Bael, S.A. & Brawn, J.D. (2005) The direct and indirect effects of insectivory by birds in two contrasting Neotropical forests. *Oecologia*, 143, 106–116.

Van Bael, S.A., Brawn, J.D. & Robinson, S.K. (2003) Birds defend trees from herbivores in a Neotropical forest canopy. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 8304–8307.

Volf, M., Klimeš, P., Lamarre, G.P.A., Redmond, C.M., Seifert, C.L., Abe, T. et al. (2019) Quantitative assessment of plant-arthropod interactions in forest canopies: a plot-based approach. *PLoS One*, 14, e0222119.

Volf, M., Weinhold, A., Seifert, C.L., Holicová, T., Uthe, H., Alander, E. et al. (2021) Branch-localized induction promotes efficacy of volatile defences and herbivore predation in trees. *Journal of Chemical Ecology*, 47, 99–111.

Volf, M., Wirth, C. & van Dam, N.M. (2020) Localized defense induction in trees: a mosaic of leaf traits promoting variation in plant traits, predation, and communities of canopy arthropods? *American Journal of Botany*, 107, 1–4.

Wold, E.N. & Marquis, R.J. (1997) Induced defense in white oak: effects on herbivores and consequences for the plant. *Ecology*, 78, 1356–1369.

SUPPORTING INFORMATION

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