



HAL
open science

Simulated Herbivory Induces Volatile Emissions of Oak Saplings, but Parasitoid Communities Vary Mainly Among Forest Sites

Freerk Molleman, Priscila Mezzomo, Manidip Mandal, Soumen Mallick, Martin Moos, Petr Vodrazka, Andreas Prinzing, Urszula Walczak

► To cite this version:

Freerk Molleman, Priscila Mezzomo, Manidip Mandal, Soumen Mallick, Martin Moos, et al.. Simulated Herbivory Induces Volatile Emissions of Oak Saplings, but Parasitoid Communities Vary Mainly Among Forest Sites. *Diversity-Basel*, 2024, 16 (12), pp.781. 10.3390/d16120781 . hal-04877387

HAL Id: hal-04877387

<https://hal.science/hal-04877387v1>

Submitted on 9 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Article

Simulated Herbivory Induces Volatile Emissions of Oak Saplings, but Parasitoid Communities Vary Mainly Among Forest Sites

Freerk Molleman ^{1,*} , Priscila Mezzomo ^{2,3} , Manidip Mandal ¹ , Soumen Mallick ⁴, Martin Moos ², Petr Vodrážka ², Andreas Prinzing ⁵  and Urszula Walczak ¹ 

¹ Department of Systematic Zoology, Institute of Environmental Biology, Faculty of Biology, Adam Mickiewicz University Poznań, Uniwersytetu Poznańskiego Str. 6, 61-614 Poznań, Poland; m.manidip1295@gmail.com (M.M.); urszulaw@amu.edu.pl (U.W.)

² Biology Centre of the Czech Academy of Sciences, 37005 Ceske Budejovice, Czech Republic; priscila.mezzomo@entu.cas.cz (P.M.); moos@bclab.eu (M.M.); vodrazka@bclab.eu (P.V.)

³ Faculty of Science, University of South Bohemia, 37005 Ceske Budejovice, Czech Republic

⁴ Field Station Fabrikschleichach, Chair of Conservation Biology and Forest Ecology, Biocenter, University of Würzburg, Glashüttenstraße 5, 96181 Rauhenebrach, Germany; soumenmallick1994@gmail.com

⁵ Research Unit «Ecosystemes, Biodiversité, Evolution», Université de Rennes 1, Centre National de la Recherche Scientifique, Campus Beaulieu, Bâtiment 14, 35042 Rennes, France; andreas.prinzing@univ-rennes1.fr

* Correspondence: fremol@amu.edu.pl; Tel.: +48-693-352-396

Abstract: We know little about how parasitoids of herbivorous insects use herbivore-induced volatile organic compounds (VOCs) to locate potential hosts on saplings in forests, and how this depends on tree composition. Therefore, we performed an experiment in a forest in Poland where we placed pairs of oak saplings (*Quercus robur* or *Q. petraea*) in neighborhoods dominated by oak, beech, or pine trees. We treated one sapling in each pair with the phytohormone methyl jasmonate, which triggers induced responses in plants. We measured the VOC emissions of thirty-six saplings and placed Malaise traps with five of the pairs. We counted the parasitoids in the ten Malaise samples and identified them using DNA metabarcoding. We used parasitoids reared from oak-feeding caterpillars to estimate which species are associated with oaks. The two species of oak differed in both the proportions of VOCs and the specific VOCs that were elevated following the application of methyl jasmonate. We did not detect any overall effects of treatment on parasitoid abundance or community composition. However, some parasitoid species that were associated with oaks appeared to be attracted to elevated emissions of specific induced VOCs. The parasitoid communities differed significantly between sites and showed marginally significant differences between neighborhoods. Overall, our results suggest that parasitoids in the understory are affected by tree composition of the canopy, but the effects of VOC emissions are limited.

Keywords: DNA metabarcoding; Malaise trap; volatile organic compounds; tree diversity



Citation: Molleman, F.; Mezzomo, P.; Mandal, M.; Mallick, S.; Moos, M.; Vodrážka, P.; Prinzing, A.; Walczak, U. Simulated Herbivory Induces Volatile Emissions of Oak Saplings, but Parasitoid Communities Vary Mainly Among Forest Sites. *Diversity* **2024**, *16*, 781. <https://doi.org/10.3390/d16120781>

Academic Editor: Luc Legal

Received: 15 November 2024

Revised: 12 December 2024

Accepted: 19 December 2024

Published: 23 December 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Parasitoids play a crucial role in controlling caterpillar populations in forests [1]. Moreover, parasitized caterpillars often exhibit much lower growth rates and likely eat less than healthy ones [2–4], so parasitoids usually also provide immediate benefits to plants. To locate their hosts, parasitoids, to a large degree, rely on volatile organic compounds (VOCs) emitted by plants in response to damage caused by herbivorous insects [5–7]. The induced response to feeding by leaf-chewing insects, such as caterpillars, is regulated by the jasmonic acid signaling pathway. Jasmonic acid and its derivatives, like methyl jasmonate (MeJA), upregulate plant defenses, including the emission of volatiles that may help natural enemies navigate towards plants that are damaged by caterpillars [8]. The attraction of

parasitoids by herbivore-induced plant volatiles has often been demonstrated in laboratory experiments (e.g., [9,10]). However, the role of VOCs in natural communities remains less understood [11–13]. This lack of understanding is probably partly due to larger spatial distances, background signals that confound observations, or abiotic factors such as wind that affect the efficiency of VOCs as cues to natural enemies of herbivores [14]. Nevertheless, Gossner et al. [15] showed that herbivore-induced VOC emissions can attract parasitoids in the understory of temperate forests. In their study, Malaise-interception traps placed next to beech saplings infested with caterpillars caught more parasitoids than those next to control saplings. However, the extent of such attraction under field conditions as well as the identity of parasitoid species that are attracted by specific VOCs remain unknown.

Many parasitoids are highly specialized to particular herbivore lineages that are specialized on particular plants and are thus also associated with specific plant species [16–20]. Therefore, their community composition can be expected to be closely linked to the local plant community [18,21–23]. Moreover, vegetation diversity can affect their efficiency in finding hosts, in part due to odor masking [24]. Notably, tree diversity can both strengthen parasitism rates at the level of the forest [25–28] and weaken it at the level of individual trees [24,29–31]. Thus, parasitoids may in part be responsible for trees suffering less herbivory when they occur in mixed stands, but this is not necessarily the rule [32]. We know even less about the role of parasitoids in determining the effect of tree diversity on saplings in the understory (see e.g., [33]). This is unfortunate because saplings may be especially vulnerable to insect herbivory and their survival, and performance affects tree recruitment [34]. Defense against herbivores often changes dramatically as plants develop, and predation pressure is often higher on trees than on saplings [35,36]. Therefore, the role of parasitoids as indirect defenses against herbivores could differ between saplings and mature trees, and thus also the effect of tree diversity on this interaction.

We investigated the effect of upregulated VOCs on parasitoids associated with oak saplings across forest sites with different canopy compositions (neighborhoods) in a forest in Poland. We first tested the prediction that parasitoid community composition in the understory is affected by canopy composition. We then specifically tested the prediction that induced saplings placed in oak-dominated neighborhoods would attract more oak-associated parasitoids than those in beech or pine neighborhoods. We further explored correlations between emissions of particular VOCs and the abundance of particular species of parasitoid associated with these saplings. We induced saplings of two oak species using MeJA and measured the VOC emissions. We then sampled the parasitoid community associated with saplings using Malaise traps. Previously, we demonstrated that our MeJA treatment induced VOC emissions in *Quercus robur* L. [37], and here we also report the emissions of *Q. petraea* (Matt.) Liebl. VOC emissions of these species have been documented before (e.g., [38–43]), and a correlation between the induction of VOCs and predation rate has been found in mature *Q. robur* [44]. VOC emissions from control and MeJA-induced saplings of these oak species were directly compared in a recent study by Mrazova et al. [45] focusing on terpenoids in oak populations in the Czech Republic. They also showed that induction increased attack rates on dummy caterpillars (mainly by birds; [45]). Expanding on that study, we present a broader analysis including other classes of VOCs in a direct comparison between the two oak species. Additionally, we examined the responses of parasitoids across multiple neighborhoods (dominated by oaks, beeches, or pines), similar to the study by Gossner's et al. [15] on beech saplings. We putatively identified parasitoid species using DNA metabarcoding. Moreover, we compared the parasitoids captured in the Malaise traps with those reared from caterpillars sampled from the crowns of oak trees in the same forest during two spring seasons. This allowed us to focus our analyses on oak-associated parasitoids, and to link the VOC emissions of individual saplings with the abundance of particular species of parasitoid.

2. Materials and Methods

2.1. Study Site

We conducted our study using *Q. robur* and *Q. petraea*. These two oak species are closely related and often hybridize [46]. They also share their herbivores, and we are not aware of a single herbivore species that would feed exclusively on one of them [47]. We placed pairs of potted oak saplings (within pairs of the same species and of similar stature; 34 *Q. robur* and 8 *Q. petraea* saplings) at a ten-meter distance from each other at sites that differed in tree species composition (neighborhood) in Puszcza Zielonka forest in Western Poland on 2 June 2022. The sites were at least 400 m apart and in different forest compartments, so we considered them independent. We used such a short distance between saplings in a pair so they would experience a very similar micro-climate and local parasitoid community. At the same time, the distance of ten meters probably ensured that they would be far enough apart to minimize direct interaction between the two saplings in terms of attracting parasitoids or communicating with each other. The tree-species composition in this forest varies markedly among compartments and within compartments, with pine (*Pinus sylvestris* L.) and sessile oak (*Q. petraea*) being the most common, followed by beech (*Fagus sylvatica* L.), hornbeam (*Carpinus betulus* L.), pedunculate oak (*Q. robur*), and spruce (*Picea abies* (L.) H.Karst; [48]). We placed our saplings at sites dominated by oak (*Q. petraea*; 52.526087, 17.053007; 52.514252, 17.062775; 52.523290, 17.058143; 52.502500, 17.064724), beech (52.509587, 17.041750; 52.501959, 17.059706), or pine (52.504600, 17.069879; 52.518787, 17.076163; 52.507266, 17.068583). This area has sandy soils with very modest small-scale relief. Tree size ranged between 40 and 100 cm DBH, and there were no naturally occurring oak saplings due to intensive browsing by deer.

2.2. Experimental Methods

We repeatedly treated one individual within each oak pair with MeJA and the other with a control solution (MeJA = 2 mM of the plant hormone MeJA, control solution = distilled water, both with molecular-grade ethanol and triton X-100; June 4, 8, 12, and 16). Saplings within each pair were of comparable size. One day after MeJA treatment, we passively sampled VOCs following Kallenbach et al. [49] with polydimethylsiloxane (PDMS) cuttings. Specifically, we placed two 1.5 cm polydimethylsiloxane tubes (PDMS; Carl Roth GmbH, Karlsruhe, Germany) on a stainless-steel wire that was attached to a twig and enclosed this twig in a polyamide bag (Stella Pack S. A. 35 × 43 cm). We attempted to enclose similar-sized twigs. After 24 h, we collected the PDMS tubes, which now contained VOCs that had been passively adsorbed from the headspace.

2.3. Analyses of VOC Samples

At the end of June 2022, we analyzed the adsorbed VOCs via thermal desorption–gas chromatography–mass spectrometry (TD–GC–MS) following the methods described in Volf et al. [50]. Specifically, we used a Thermal Desorption (TD) TD100-xr (Markes International, Bridgend, UK) installed on a Trace 1300 gas chromatograph coupled to an ISQ quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). We inserted the PDMS tubes into metal tubes and placed them in the TD instrument. We then dried the tube with a helium flow (20 mL per minute) for five minutes before heating it to 200 °C for five minutes with a flow of 60 mL per minute. We captured the desorbed VOCs in a cold trap of 0 °C, which we then heated to 300 °C for four minutes with a split flow of 10 mL per minute. We performed GC separation using a VF-5MS capillary column (30 m × 0.25 mm id, 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA, USA) with helium as the carrier gas at a flow rate of 2 mL per minute. The temperature conditions we used were as follows: 39 °C for one minute, increasing at a rate of 8 °C per minute to 95 °C, and then increasing to 300 °C at a rate of 20 °C per minute. Finally, this temperature was held for two minutes. We detected the VOCs using an electron ionization (EI) ion source (70 eV, 230 °C) and a single quadrupole in a scan range of 33–500 *m/z*. We used Thermo Xcalibur 2.0 software (Qual Browser, Quan Browser, Thermo Fisher Scientific)

for data processing, method calibration, and validation. We quantified VOC emissions as the area under the chromatographic peak using Bruker Workstation software (v.8.0.1; more details in [37]). We tentatively identified the detected VOCs through comparisons to acquired standards, the NIST20 database (National Institute of Standards and Technology, Gaithersburg, MD, USA), and comparisons to retention indices from the literature.

2.4. Sampling of Parasitoids

For the five pairs that included the smallest oak saplings, we placed Malaise traps (Paradox Company, Kraków, Poland) over the saplings to intercept insects flying in their vicinity (Figure 1). Other saplings were too tall to fit under the traps. Among these, three pairs consisted of *Q. petraea* saplings, and two pairs of *Q. robur* saplings. On 10 June, we collected the first Malaise sample from the traps. To minimize positional effects, we then switched the positions of the saplings within each pair of Malaise traps. We collected the second and last Malaise samples on 20 June. Therefore, each trap operated for 18 days during the peak of caterpillar abundance [51,52], ten or eight days for each sapling in a pair. As a result, we collected two samples per trap, and we later combined data from samples according to the sapling that was with a trap, taking into account that we switched the saplings after the first sample was taken from the trap. Insects were preserved in ca. 95% ethanol and stored at -20°C . To sample parasitoids associated with the other saplings, we placed a dark-green sticky trap with each of the remaining saplings, but this was not successful.



Figure 1. A pair of Malaise traps placed over oak saplings in Puszcza Zielonka forest, Poland. The sapling is situated behind the central screen.

2.5. Estimating Parasitoid Community Composition

To provisionally identify the parasitoid communities from the Malaise samples, we used DNA metabarcoding. All parasitoid wasps, along with some visually similar Hymenoptera, were extracted from the samples, sorted according to their size (large vs. small), and counted. Samples of the same size class were usually combined for the two sampling dates for a given sapling, resulting in samples containing between 7 and 58 individuals

(average: 33). Sorting by size is recommended because in meta-barcoding, the signals of some large-bodied specimens can overwhelm the signals of small-bodied specimens [53]. Additionally, to build a reference library of parasitoids that target caterpillars feeding on oak leaves, we pooled parasitoids reared from caterpillars collected during the springs of 2019 [52] and 2021 [54]. These parasitoids were also sorted into small- and large-bodied species categories, and DNA metabarcoding was performed on these two samples. Notably, the caterpillars from which they were reared were not identified to species, and parasitoids were mixed for metabarcoding, so we do not know from which hosts these parasitoids were reared, only that their hosts fed on oak.

Species identification of parasitoids followed the laboratory and bioinformatic pipelines reported by Uhler et al. [55]. In summary, after removing the ethanol, the samples were dried at 65 °C in an oven overnight in 20 mL glass vials and then homogenized using stainless steel beads within a FastPrep 96 instrument (MP Biomedicals, Santa Ana, CA, USA). We estimated the total mass of the samples to be between 200 and 700 mg of biomass. DNA extraction was performed on all samples using a 90:10 solution of animal lysis buffer (buffer ATL, Qiagen DNEasy Tissue Kit with 10% proteinase K, Qiagen, Hilden, Germany). Lysis was carried out overnight at 56 °C, followed by cooling to room temperature in a total volume of 1 mL. DNA extraction from 200 µL aliquots of the lysate was performed using a DNEasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions, and the residual lysate was then discarded. From each sample, 5 µL of extracted genomic DNA was used for PCR amplification with Plant MyTAQ (Bioline, Luckenwalde, Germany), and high-throughput sequencing (HTS) adapted mini-barcode primers targeting the mitochondrial CO1-5P region, following Leray et al. [56]. One PCR was performed for each sample. Amplification success and fragment length were assessed using gel electrophoresis. A clean-up of the amplified DNA and Illumina Nextera XT (Illumina Inc., San Diego, CA, USA) index ligations was performed following standard protocols. DNA concentrations were quantified using a Qubit fluorometer (Life Technologies, Carlsbad, CA, USA), and the samples were combined into equimolar pools. The pooled DNA was purified using NGS magnetic beads (MagSi-NGSprep Plus Magtivio). HTS was performed on an Illumina MiSeq using v3 chemistry (2 × 300 bp, 600 cycles, maximum of 25 mio paired-end reads). The sequencing data were processed using the VSEARCH suite v2.9.1 and Cutadapt K5. Quality filtering and chimera removal were performed, and the remaining sequences were clustered into operational taxonomic units (OTUs) at 97% identity. OTUs were then assigned to barcode index numbers (BINs) using the BOLD database [57]. A cleaning step was carried out to exclude low-abundance OTUs. Additionally, a separate BLAST search against the NCBI nucleotide database was conducted to validate the BOLD results.

2.6. Data Analysis

To test whether VOC emissions were affected by MeJA treatment in a species-specific manner, we first log-transformed the VOC emissions to approach a normal distribution of residuals. To investigate if specific VOCs were upregulated upon induction, we implemented one-way ANOVAs for each VOC. Subsequently, we visualized the similarity of VOC emission among saplings using non-metric multidimensional scaling (NMDS) and implemented PERMANOVA models. Species (*Q. robur* vs. *Q. petraea*), treatment (MeJA sprayed vs. control), and their interaction were included as predictors in the PERMANOVA analysis.

DNA metabarcoding generated a large dataset with numerous barcode index numbers (BINs) and species identified across samples, displaying signals (yield) ranging from very strong to very weak (Supplementary Material Table S1). To translate these data into putative parasitoid communities for each trap, we began by removing non-parasitoid species from the dataset. We then standardized the species nomenclature to achieve consistency, resorting to genus or family levels where necessary, and assigned numbers to species within their clade. Furthermore, we resolved cases of duplicate species identification caused by slight barcode variations by combining counts across affected samples. Next,

we ensured that the number of identified species within a sample matched the number of individuals present. To achieve this, we calculated the average yield per individual by dividing the summed yield of parasitoid taxa by the number of individual parasitoids in the sample. We then removed any species with signals weaker than 10% of the average yield per individual. Using the remaining data, we estimated individual counts per species by assigning yields proportionately, ensuring that the total count aligned with the initially recorded number of individuals. This allocation process assumed equal yields across species. However, to compensate for those with low yields, which we assigned one individual, we had to assign those species with high yields fewer individuals than they would otherwise have been assigned (Supplementary Material Table S1). Finally, we combined the parasitoid community estimates from each sample (large and small body sizes) to derive the estimated parasitoid community for each sapling. We treated parasitoids from the caterpillars reared from oak trees similarly to those from Malaise traps, providing a list of oak-associated parasitoids, but not estimating their abundances. We used this list to create a subset of the trapping data containing only those parasitoid species confirmed to be associated with oak-feeding caterpillars (hereafter referred to as oak-associated parasitoids). To assess the degree of sample completeness of the parasitoid communities, we constructed a rarefaction plot.

To test whether MeJA treatment affected overall parasitoid abundance, we performed paired *t*-tests within pairs of saplings. To gain insight into parasitoid community composition, we visualized the similarities of their communities associated with saplings using non-metric multi-dimensional scaling (NMDS) and performed PERMANOVA analyses. First, we tested for the effects of both neighborhood and treatment as well as their interaction using regular PERMANOVA, excluding the interaction term if it was non-significant. Second, we tested separately for the effect of site, as within a given site, there can only be one type of neighborhood. Third, we tested for the effect of treatment while accounting for the paired setup of traps within sites using paired PERMANOVA. We repeated these analyses for the oak-associated parasitoids and for those that were not shown to be associated with oaks. Additionally, we explored whether specific parasitoid species are attracted to specific VOCs using a correlogram. We focused on the oak-associated species of which we caught at least four individuals, and correlated their abundance with log emissions of each of the nineteen identified VOCs using Pearson correlation. We plotted the results as a heatmap, highlighting VOCs that were upregulated upon induction for each species of oak. We performed all analyses and visualizations in the R statistical environment version 4.4.0 using the packages *vegan* and *ggplot2* [58–60].

3. Results

3.1. VOC Emissions

We identified 19 VOCs emitted by *Q. petraea* and *Q. robur* saplings, two of which ((Z,E)- α -Farnesene and α -Farnesene) were significantly upregulated in MeJA-treated *Q. petraea* saplings (Table 1). Although the significance would disappear after correcting for multiple testing, the effect size appeared large (note log scale). These differed from the upregulated compounds in *Q. robur*, which included Eucalyptol, Linalool, o-Cymene, β -Ocimene, β -cis-Ocimene, and α -Copaene (Table 1). While the overall VOC emission profiles of the two species overlapped (Figure 2), they still showed significant differences (Table 2). Additionally, there was a marginally significant interaction between species and treatment (Table 2), as the VOCs elevated upon induction differed between the two species of oak (Table 1; [37]).

Table 1. Results of one-way ANOVAs for effect of treatment (control vs. MeJA application) on log of volatile organic compound (VOC) emissions of 8 *Quercus petraea* and 34 *Q. robur* saplings, with means, where $p < 0.05$ is in bold font and $p < 0.1$ is underlined. All effects of MeJA were positive. Results for *Q. robur* were published previously in [37].

Class	<i>Q. petraea</i> Variable					<i>Q. robur</i>			
		MeJA	Control	F-Value	<i>p</i> -Value	MeJA	Control	F-Value	<i>p</i> -Value
Aldehydes	Nonanal	16.2	16.3	0.002	0.965	17.4	16.4	5.788	0.023
Alkanes	Eicosane	16.9	16.6	0.047	0.837	16.8	16.4	1.153	0.292
	Hexadecane	16.2	15.4	0.303	0.606	15.9	15.7	0.227	0.638
	Pentadecane	16.2	15.4	0.303	0.606	15.9	15.7	0.227	0.638
	Tridecane	15.3	15.2	0.010	0.925	15.6	15.1	1.657	0.209
Green Leaf Volatiles	Methyl salicylate	15.7	15.9	0.008	0.932	17.1	16.1	4.202	0.05
Monoterpenes	Eucalyptol	17.7	15.8	2.460	0.178	16.9	14.6	21.857	<0.001
	Limonene	15.3	15.0	0.256	0.634	15	14.6	4.28	0.048
	Linalool	17.2	15.3	2.487	0.176	16.7	14.7	36.715	<0.001
	o-Cymene	16.3	13.6	3.929	0.104	15.5	13.6	16.896	<0.001
	β -Ocimene	18.5	14.9	5.907	<u>0.059</u>	18.3	14.8	28.03	<0.001
	β -cis-Ocimene	15.5	13.8	1.201	0.323	15.7	13.6	25.563	<0.001
Sesquiterpenes	(Z,E)- α -Farnesene	19.3	16.2	7.025	0.045	18.2	16.6	6.21	0.019
	α -Farnesene	19.3	16.2	6.981	0.046	18.2	16.6	6.305	0.018
	Caryophyllene	18.8	16.6	5.374	<u>0.068</u>	17.9	17.1	3.372	0.077
	α -Copaene	16.2	14.4	2.487	0.176	15.6	14.4	31.089	<0.001
	α -Cubebene	16.0	14.5	3.331	0.128	15.6	14.8	3.553	0.07
	δ -Cadinene	16.9	15.1	5.172	<u>0.072</u>	17.1	15.7	8.782	0.006

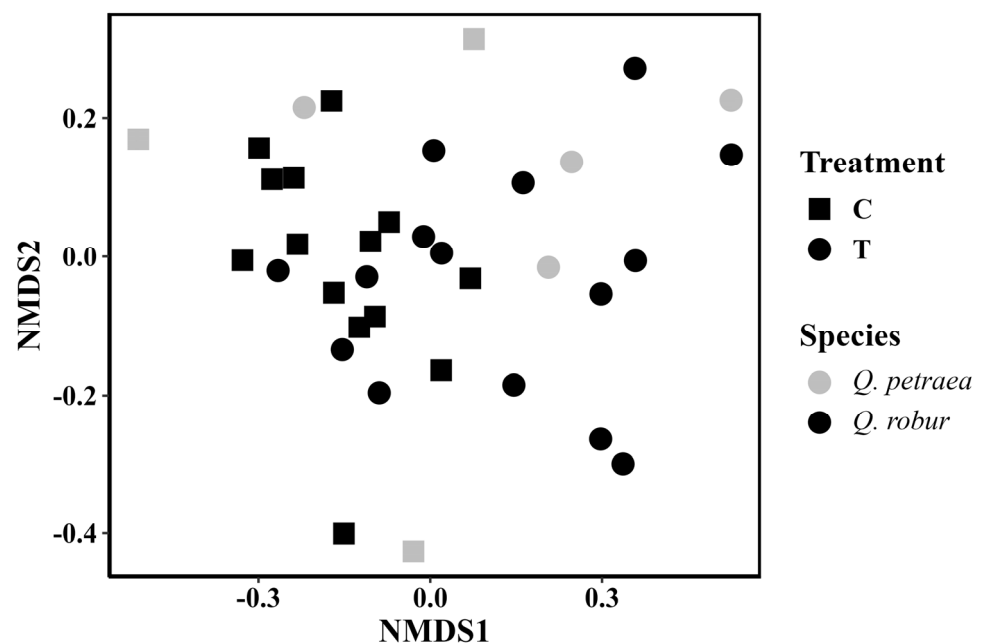


Figure 2. Non-metric multi-dimensional scaling (NMDS) plots of log volatile organic compound (VOC) emissions of oak saplings by MeJA treatment (C = control; T = MeJA treatment) and species (*Q. petraea* vs. *Q. robur*). Statistical results are given in Table 2.

Table 2. PERMANOVA results for log VOC emissions of oak saplings. Species = *Q. robur* or *Q. petraea*; Treatment = MeJA application vs. control.

	Df	SoS	R2	F-Value	<i>p</i> -Value
Species	1	0.436	0.053	2.27	0.036
Treatment	1	1.207	0.147	6.27	0.001
Species:Treatment	1	0.391	0.048	2.03	<u>0.057</u>
Residual	32	6.157	0.752		
Total	35	8.191	1		

3.2. Parasitoids

The DNA metabarcoding of the Malaise trap samples and our data processing pipeline determined that the 963 parasitoids collected belonged to 244 putative species. These parasitoids belonged to ten families (Braconidae (147 putative individuals, 63 putative species), Diapriidae (70, 30), Dryinidae (2, 2), Eulophidae (1, 1), Evaniidae (17, 1), Ichneumonidae (646, 127), Megastigmidae (2, 1), Ormyridae (2, 1), Proctotrupidae (4, 4), and Pteromalidae (7, 5)), and 65 individuals belonging to 14 species were not assigned to a family (Supplementary Material Table S1). The number of individuals associated with a sapling ranged from 76 to 112, encompassing between 27 and 48 species. The rarefaction curve shows that we sampled only a small fraction of the parasitoid species in this forest (Figure 3). Among the parasitoids that were reared from oak-feeding caterpillars during previous years, 182 species were detected using DNA metabarcoding. These formed our reference collection. Of these species, 82 were also found in the Malaise trap samples, representing 398 individuals. This undoubtedly unjustly excluded some oak-associated species that were not represented in the reared sample. Very few parasitoids were captured in sticky traps.

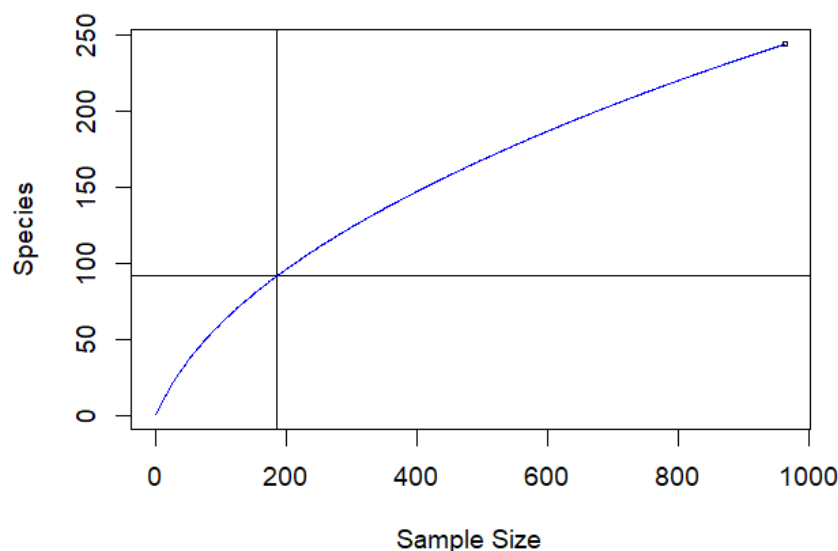


Figure 3. Rarefaction curve for parasitoids collected with ten Malaise traps in Puszcza Zielonka forest in June 2022. The blue line shows the number of species that was represented for a given number of individuals (sample size) collected.

There were no significant differences between the control and MeJA-treated oak saplings in the number of parasitoids captured in the Malaise traps (paired *t*-test: $t = 0.21$, $p = 0.41$), the number of oak-associated parasitoids (paired *t*-test: $t = -0.60$, $p = 0.29$), or those not shown to be associated with oaks (paired *t*-test: $t = 1.03$, $p = 0.18$). Likewise, the community composition was not significantly affected by MeJA treatment (Table 3, Figure 4). Nevertheless, the abundance of a few putative species of parasitoid appeared to be positively correlated with the emission of specific VOCs (Figure 5). Notably, Ichneumonid 13 was associated with elevated emissions of VOCs characteristic of induced

Q. petraea ((Z,E)- α - and α -Farnesene), although this was due to all six individuals being found in a single trap. VOCs associated with induced *Q. robur* saplings (Eucalyptol, Linalool, o-Cymene, β -Ocimene, β -cis-Ocimene, and α -Copaene) appeared to attract the parasitoids *Meteorus 2*, and *Exochus 1* (Figure 5). Additionally, some Braconidae and Ichneumonid 10 seemed to be attracted to alkanes that are not associated with MeJA induction of these oaks (Figure 5). Some species were associated with lower emissions of certain VOCs (Figure 5). Overall, putative parasitoid communities differed significantly between sites and marginally significantly between neighborhoods (Figure 4a, Table 3). Oak-associated putative parasitoid communities differed significantly between sites, but not between neighborhoods (Figure 4b, Table 3).

Table 3. PERMANOVA results of parasitoid communities in paired Malaise traps (five pairs, ten traps in total) as determined using DNA metabarcoding. In paired PERMANOVA, communities were compared across pairs of saplings within sites. Statistically significant *p*-values (*p* < 0.05) are in bold font and marginally significant ones (*p* < 0.1) are underlined. This table is illustrated in Figure 4.

Predictor	Df	SoS	All Parasitoids			Those Not Associated with Oaks				Oak-Associated Parasitoids			
			R2	F-Value	<i>p</i> -Value	SoS	R2	F-Value	<i>p</i> -Value	SoS	R2	F-Value	<i>p</i> -Value
Treatment	1	0.305	0.088	0.73	0.792	0.296	0.086	0.737	0.817	0.369	0.1	0.821	0.654
Neighbourhood	2	0.984	0.283	1.18	0.195	0.974	0.283	1.213	0.174	0.966	0.262	1.074	0.328
Treatment*Neighbourhood	2	0.517	0.149	0.62	0.983	0.56	0.163	0.697	0.934	0.547	0.149	0.608	0.97
Residual	4	1.668	0.48			1.606	0.467			1.8	0.489		
Total	9	3.474				3.436				3.682			
Treatment		0.305	0.088	0.836	0.689	0.296	0.086	0.819	0.701	0.369	0.1	0.944	0.552
Neighbourhood	2	0.984	0.283	1.352	<u>0.071</u>	0.974	0.283	1.349	<u>0.084</u>	0.966	0.262	1.235	0.179
Residual	6	2.185	0.629			2.167	0.63			2.347	0.637		
Total	9	3.474				3.436				3.682			
Treatment	1	0.305	0.088	1.092	0.421	29.60%	0.086	1.03	0.44	0.369	0.1	1.245	0.263
Site	4	2.054	0.591	1.341	0.007	1.991	0.579	1.733	0.005	2.127	0.577	1.792	0.026
Residual	4	1.115	0.321			1.149	0.334			1.187	0.322		
Total	9	3.474				3.436				3.682			
Paired Malaise traps within sites													
Treatment	1	0.305	0.088	0.769	0.375	0.296	0.086	0.754	0.5	0.369	0.088	0.892	0.313
Residual	8	3.169	0.912			3.141	0.914			3.313	0.9		
Total	9	3.474				3.436				3.682			

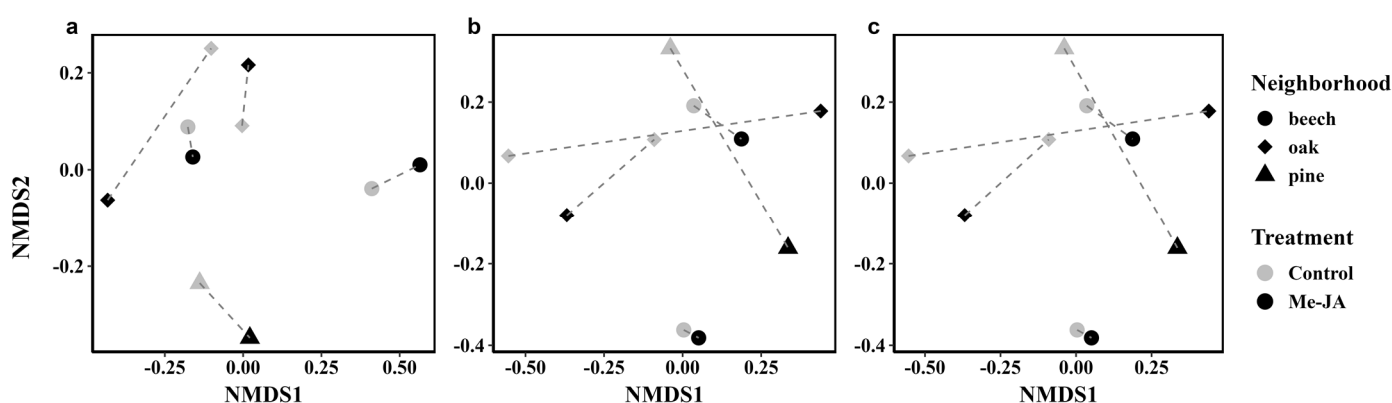


Figure 4. NMDS results for (a) all parasitoids, (b) parasitoids not shown to be associated with oaks, and (c) parasitoids associated with oaks, captured in Malaise traps placed over oak saplings. Parasitoids caught at each sapling were counted and then identified using DNA metabarcoding. The NMDS axes were calculated separately for each plot, and oak saplings in a pair are connected with dashed lines. Statistical results are given in Table 3.

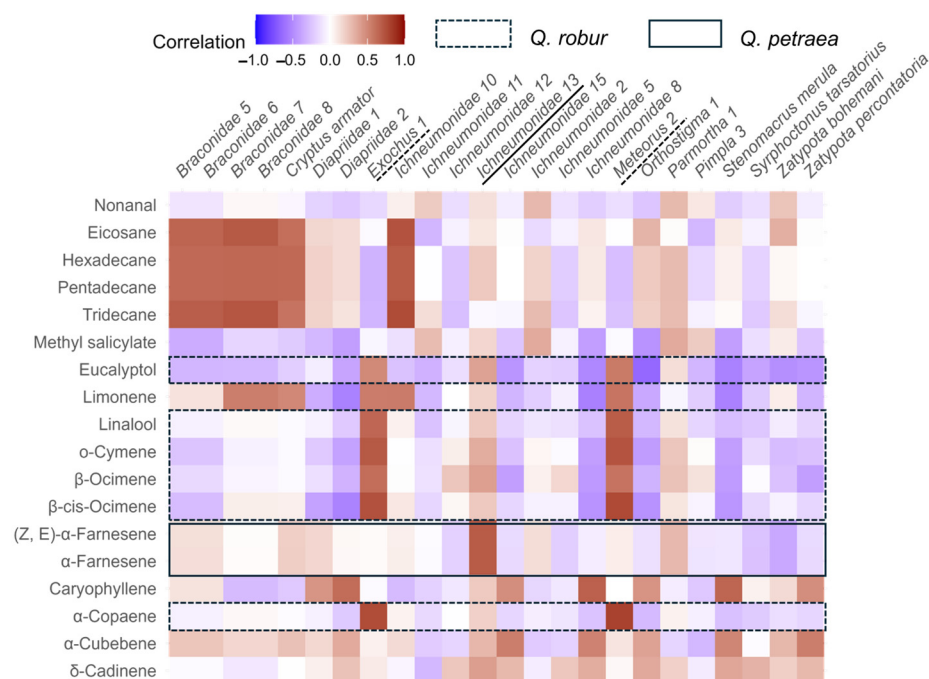


Figure 5. An exploration of the Pearson correlation coefficients between the log-transformed VOC emissions and the number of individuals of the most commonly intercepted oak-associated parasitoids across ten saplings placed in a forest in Poland. VOCs that are elevated upon induction are marked for each species of oak with boxes (dotted or continuous lines, depending on the species), and parasitoid species that appear associated with these VOCs are underlined in the matching style.

4. Discussion

We placed oak saplings in pairs across three different neighborhoods to identify which VOC emissions are elevated after induction and to gauge their effects on parasitoids in neighborhoods with different canopy compositions. Although the identified VOCs overlapped between the two oak species, their proportions differed, and they responded differently to induction. The number of parasitoids and their provisional community composition did not differ significantly between induced and control saplings. However, the abundance of some parasitoid species appeared to correlate with VOC emissions induced in specific oak species. Moreover, we found significant differences in parasitoid communities across sites, which seemed to be linked to tree-species composition.

Both studied oak species produced the same VOCs. The differences occurred mainly in their relative proportions following induction, which is in line with the findings of other studies across a broad spectrum of oak species [39]. Our findings thus corroborate the results of Mrazova et al. [45], who studied the same oak species. However, the identities of the main upregulated VOCs reported in this study differed from those recorded here. For example, Mrazova et al. [45] reported β -ocimene as the main compound elevated in induced *Q. petraea* saplings, while this compound was only marginally significantly elevated in our study. Moreover, we found a significant increase in the emissions of (Z,E)- α - and α -Farnesene in *Q. petraea*, not reported by Mrazova et al. [45]. This points to substantial trait variation between the studied oaks. Such trait differences between co-occurring related plants are common [61] and may be related to selection for niche partitioning. Previous studies showed that oaks exhibit pronounced differences in their chemistry even at the population level (e.g., [62]). This variability may help them to cope with variable herbivory and other conditions they face [63]. Additionally, these differences may also reflect the environmental conditions at the two sites. While both studies were performed in similar habitats and during the same time of year, it is possible that differences in temperature, humidity, or light conditions affected the emissions of VOCs (e.g., [64–66]). Different induced signals could potentially serve to attract specialized natural enemies of

herbivores [67]. However, this would be surprising in this particular system, as most insects that feed on one of these species of oak also feed on the other (see [68] for an example of the effect of tree species on parasitism in one host species). Whether the specificity in induced VOCs between these oak species affects their interactions with parasitoids thus warrants further study.

Despite parasitoids being known for homing in on induced VOCs when searching for hosts (e.g., [69]), we were unable to detect the overall effects of induction on provisional parasitoid communities in our Malaise traps, and the sticky traps collected few parasitoids. Nevertheless, correlations between individual VOCs and the abundance of certain species of parasitoid in traps suggest some instances of attraction that warrant further study. These might represent cases where particular parasitoid species use particular VOCs to find their host. With a larger number of saplings with VOC emission and parasitoid community data, we could test the robustness of such correlations, and controlled experiments could further test if these VOCs are indeed attracting these particular parasitoids. The lack of overall effects of induction contrast with the findings of Gossner et al. [15], who performed a similar study on beech saplings. This discrepancy may be attributable to our smaller number of traps (10 compared to 36 used by Gossner et al. [15]), the small size of the saplings, and the use of artificial induction rather than using caterpillars. Additionally, captures in Malaise traps can vary greatly between seemingly similar trap positions, likely due to small differences in microhabitat, making Malaise trap data noisy. We tried to mitigate this issue by placing saplings in pairs and switching their positions in the middle of the experiment. However, saplings were in different positions at different times, so their position could still affect the catch. Furthermore, MeJA triggers strong but largely unspecific responses [41]. In contrast, VOC signals emitted upon herbivory by real insect herbivores seem to be highly specific [70,71]. This specificity can then play an important role in attracting parasitoids that are largely specialized themselves [72]. Moreover, VOC emissions change over time [45,73], and we only measured VOC emissions once, while saplings were induced multiple times and the traps operated for 20 days. Nonetheless, the VOC response was marked, resulting in increased predation rates on dummy caterpillars in other studies on these plant species [44,45], and the attraction of parasitoids in other systems (e.g., [74]). Overall, we may surmise that there were no overwhelming effects of the induction of oak saplings on parasitoid attraction. However, with a larger sample size and more natural induction, we would perhaps observe a larger number of significant effects for particular species of parasitoid.

We found a significant effect of the site where the saplings were placed on the composition of parasitoid communities. Presumably, the site effect is mainly determined by the local parasitoid community, but it may partly be caused by differences in sapling size between pairs. The site effect seemed to be linked, at least partially, to tree species in the canopy, but this was not significant. Our findings align with some previous studies exploring the impact of forest composition on understory insect communities (e.g., [33]). However, our results contrast with those of Gossner et al. [15], where the abundance and diversity of trapped parasitoids were not related to the number of naturally occurring beech saplings. This may suggest that parasitoids are highly mobile and disperse widely [75–77]. On the other hand, Gossner et al. [15] used a highly polyphagous caterpillar to induce saplings, and thus, its parasitoids may also occur in association with many species of tree. Their results might thus have been different had they used a more specialized caterpillar. Our results suggest that even within a patchy forest where oak trees are abundant, the composition of parasitoid communities in the understory is influenced by the species composition of trees in the canopy at a fine spatial scale. Notably, we only captured a small proportion of the parasitoid community, indicating very high local richness, similar to other Malaise trap studies in forests [78]. Moreover, most species remained unidentified using DNA metabarcoding, further emphasizing this large gap in our knowledge on relatively well-studied European temperate forests. Finally, we point out that while sticky traps could provide a cost-effective alternative to Malaise traps, species identification might be

more challenging, and we observed minimal parasitoid captures with them. Transparent or light-colored sticky traps or yellow pan traps might be more effective for sampling parasitoids compared to the dark sticky traps we used.

5. Conclusions

We observed notable effects of MeJA induction on VOC emissions, but did not find clear evidence of its effect on parasitoid communities. Our study highlights the influence of canopy tree species composition in shaping parasitoid communities in the understory. Further studies are needed to understand how different parasitoid lineages move in the landscape and how they respond to canopy composition, VOC profiles, and plant size [14,28,77].

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d16120781/s1>, Table S1: Meta barcodes Parasitoids Data.

Author Contributions: F.M. developed the initial concept of the study, led the fieldwork, sorted the parasitoids, analyzed the data, and wrote the first draft of the manuscript. P.M. participated in developing the concept and provided training in VOC sample collection and the interpretation of VOC measurements. M.M. (Manidip Mandal) participated in the planning and fieldwork, performed induction, collected the VOC samples, and participated in the VOC analysis. S.M. participated in the fieldwork and collection of VOC samples. M.M. (Martin Moos) and P.V. advised on VOC sample collection and performed VOC measurements. A.P. participated in developing the concept. U.W. participated in developing the concept, planning the study, and fieldwork. All authors contributed to writing the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by grant No. 2018/29/B/NZ8/00112 awarded to Freerk Molleman from the National Science Centre (NCN, Poland), and Priscila Mezzomo acknowledges support from the Grant Agency of the Czech Republic (grant number 23-06855L).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: We provide an Excel file as an appendix with the metabarcoding data.

Acknowledgments: Fieldwork was carried out with the permission of the Forestry Experimental Station in Murowana Goślina (Poznań University of Life Sciences). We are grateful to Fatmanur Selvi for her help in the field and Martin Volf for help with the study design and editing the manuscript. The DNA metabarcoding was performed by AIM—Advanced Identification Methods GmbH, Leipzig, Germany.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Stireman, J.O.; Shaw, S.R. Natural History and Ecology of Caterpillar Parasitoids. In *Caterpillars in the Middle: Tritrophic Interactions in a Changing World*; Marquis, R.J., Koptur, S., Eds.; Springer International Publishing: Cham, Switzerland, 2022; pp. 225–272.
2. Alleyne, M.; Beckage, N.E. Parasitism-induced effects on host growth and metabolic efficiency in tobacco hornworm larvae parasitized by *Cotesia congregata*. *J. Insect Physiol.* **1997**, *43*, 407–424. [[CrossRef](#)]
3. Moore, M.E.; Kester, K.M.; Kingsolver, J.G. Rearing temperature and parasitoid load determine host and parasitoid performance in *Manduca sexta* and *Cotesia congregata*. *Ecol. Entomol.* **2020**, *45*, 79–89. [[CrossRef](#)]
4. Cuny, M.A.; Poelman, E.H. Evolution of koinobiont parasitoid host regulation and consequences for indirect plant defence. *Ecol. Ecol.* **2022**, *36*, 299–319. [[CrossRef](#)]
5. Sobhy, I.; Tamiru, A.; Chiriboga Morales, X.; Nyagol, D.; Cheruiyot, D.; Chidawanyika, F.; Subramanian, S.; Midega, C.; Bruce, T.; Khan, Z. Bioactive volatiles from push-pull companion crops repel fall armyworm and attract its parasitoids. *Front. Ecol. Evol.* **2022**, *10*, 883020. [[CrossRef](#)]
6. Janssen, A.; Sabelis, M.W.; Bruin, J. Evolution of herbivore-induced plant volatiles. *Oikos* **2002**, *97*, 134–138. [[CrossRef](#)]
7. Dicke, M.; Lucas-Barbosa, D. Herbivore-induced plant volatiles as a source of information in plant–insect networks. In *Biology of Plant Volatiles*; CRC Press: Boca Raton, FL, USA, 2020; pp. 327–346.
8. Lortzing, T.; Steppuhn, A. Jasmonate signalling in plants shapes plant–insect interaction ecology. *Curr. Opin. Insect Sci.* **2016**, *14*, 32–39. [[CrossRef](#)] [[PubMed](#)]
9. Fatouros, N.E.; van Loon, J.J.A.; Hordijk, K.A.; Smid, H.M.; Dicke, M. Herbivore-induced plant volatiles mediate in-flight host discrimination by parasitoids. *J. Chem. Ecol.* **2005**, *31*, 2033–2047. [[CrossRef](#)] [[PubMed](#)]

10. Girling, R.D.; Stewart-Jones, A.; Dherbecourt, J.; Staley, J.T.; Wright, D.J.; Poppy, G.M. Parasitoids select plants more heavily infested with their caterpillar hosts: A new approach to aid interpretation of plant headspace volatiles. *Proc. R. Soc. B Biol. Sci.* **2011**, *278*, 2646–2653. [[CrossRef](#)] [[PubMed](#)]
11. Gish, M.; De Moraes, C.M.; Mescher, M.C. Herbivore-induced plant volatiles in natural and agricultural ecosystems: Open questions and future prospects. *Curr. Opin. Insect Sci.* **2015**, *9*, 1–6. [[CrossRef](#)]
12. Fernandez-Conradi, P.; Castagnyrol, B.; Jactel, H.; Rasmann, S. Combining phytochemicals and multitrophic interactions to control forest insect pests. *Curr. Opin. Insect Sci.* **2021**, *44*, 101–106. [[CrossRef](#)] [[PubMed](#)]
13. Aartsma, Y.; Bianchi, F.; van der Werf, W.; Poelman, E.H.; Dicke, M. Herbivore-induced plant volatiles and tritrophic interactions across spatial scales. *New Phytol.* **2017**, *216*, 1054–1063. [[CrossRef](#)]
14. Douma, J.C.; Ganzeveld, L.N.; Unsicker, S.B.; Boeckler, G.A.; Dicke, M. What makes a volatile organic compound a reliable indicator of insect herbivory? *Plant Cell Environ.* **2019**, *42*, 3308–3325. [[CrossRef](#)]
15. Gossner, M.M.; Weisser, W.W.; Gershenson, J.; Unsicker, S.B. Insect attraction to herbivore-induced beech volatiles under different forest management regimes. *Oecologia* **2014**, *176*, 569–580. [[CrossRef](#)]
16. Godfray, H.C.J. *Parasitoids: Behavioral and Evolutionary Ecology*; Princeton University Press: Princeton, NJ, USA, 1994; Volume 12.
17. van Oudenhove, L.; Mailleret, L.; Fauvergue, X. Infochemical use and dietary specialization in parasitoids: A meta-analysis. *Ecol. Evol.* **2017**, *7*, 4804–4811. [[CrossRef](#)] [[PubMed](#)]
18. Šigut, M.; Šigutová, H.; Šipoš, J.; Pyszko, P.; Kotásková, N.; Drozd, P. Vertical canopy gradient shaping the stratification of leaf-chewer–parasitoid interactions in a temperate forest. *Ecol. Evol.* **2018**, *8*, 7297–7311. [[CrossRef](#)] [[PubMed](#)]
19. Hrcek, J.; Miller, S.E.; Whitfield, J.B.; Shima, H.; Novotny, V. Parasitism rate, parasitoid community composition and host specificity on exposed and semi-concealed caterpillars from a tropical rainforest. *Oecologia* **2013**, *173*, 521–532. [[CrossRef](#)]
20. Smith, M.A.; Rodriguez, J.J.; Whitfield, J.B.; Deans, A.R.; Janzen, D.H.; Hallwachs, W.; Hebert, P.D.N. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 12359–12364. [[CrossRef](#)]
21. Fraser, S.E.; Dytham, C.; Mayhew, P.J. Determinants of parasitoid abundance and diversity in woodland habitats. *J. Appl. Ecol.* **2007**, *44*, 352–361. [[CrossRef](#)]
22. Rodriguez, A.; Pohjoismäki, J.L.; Kouki, J. Diversity of forest management promotes parasitoid functional diversity in boreal forests. *Biol. Conserv.* **2019**, *238*, 108205. [[CrossRef](#)]
23. Peralta, G.; Frost, C.M.; Didham, R.K. Plant, herbivore and parasitoid community composition in native Nothofagaceae forests vs. exotic pine plantations. *J. Appl. Ecol.* **2018**, *55*, 1265–1275. [[CrossRef](#)]
24. Jactel, H.; Birgersson, G.; Andersson, S.; Schlyter, F. Non-host volatiles mediate associational resistance to the pine processionary moth. *Oecologia* **2011**, *166*, 703–711. [[CrossRef](#)] [[PubMed](#)]
25. Staab, M.; Bruelheide, H.; Durka, W.; Michalski, S.; Purschke, O.; Zhu, C.-D.; Klein, A.-M. Tree phylogenetic diversity promotes host–parasitoid interactions. *Proc. R. Soc. B Biol. Sci.* **2016**, *283*, 20160275. [[CrossRef](#)] [[PubMed](#)]
26. Guo, P.-F.; Wang, M.-Q.; Orr, M.; Li, Y.; Chen, J.-T.; Zhou, Q.-S.; Staab, M.; Fornoff, F.; Chen, G.-H.; Zhang, N.-L. Tree diversity promotes predatory wasps and parasitoids but not pollinator bees in a subtropical experimental forest. *Basic Appl. Ecol.* **2021**, *53*, 134–142. [[CrossRef](#)]
27. Haddad, N.M.; Crutsinger, G.M.; Gross, K.; Haarstad, J.; Knops, J.M.; Tilman, D. Plant species loss decreases arthropod diversity and shifts trophic structure. *Ecol. Lett.* **2009**, *12*, 1029–1039. [[CrossRef](#)]
28. Moreira, X.; Abdala-Roberts, L.; Rasmann, S.; Castagnyrol, B.; Mooney, K.A. Plant diversity effects on insect herbivores and their natural enemies: Current thinking, recent findings, and future directions. *Curr. Opin. Insect Sci.* **2016**, *14*, 1–7. [[CrossRef](#)] [[PubMed](#)]
29. Yguel, B.; Bailey, R.I.; Villemant, C.; Brault, A.; Jactel, H.; Prinzing, A. Insect herbivores should follow plants escaping their relatives. *Oecologia* **2014**, *176*, 521–532. [[CrossRef](#)] [[PubMed](#)]
30. Mallick, S.; Molleman, F.; Yguel, B.; Bailey, R.; Müller, J.; Jean, F.; Prinzing, A. Ectophagous folivores do not profit from rich resources on phylogenetically isolated trees. *Oecologia* **2022**, *201*, 1–18. [[CrossRef](#)] [[PubMed](#)]
31. Hidasi-Neto, J.; Bailey, R.I.; Vasseur, C.; Woas, S.; Ulrich, W.; Jambon, O.; Santos, A.M.C.; Cianciaruso, M.V.; Prinzing, A. A forest canopy as a living archipelago: Why phylogenetic isolation may increase and age decrease diversity. *J. Biogeogr.* **2019**, *46*, 158–169. [[CrossRef](#)]
32. Jactel, H.; Moreira, X.; Castagnyrol, B. Tree diversity and forest resistance to insect pests: Patterns, mechanisms, and prospects. *Annu. Rev. Entomol.* **2021**, *66*, 277–296. [[CrossRef](#)]
33. Setiawan, N.N.; Vanhellefont, M.; Baeten, L.; Gobin, R.; De Smedt, P.; Proesmans, W.; Ampoorter, E.; Verheyen, K. Does neighbourhood tree diversity affect the crown arthropod community in saplings? *Biodivers. Conserv.* **2016**, *25*, 169–185. [[CrossRef](#)]
34. Crawley, M.J. Insect herbivores and plant population dynamics. *Annu. Rev. Entomol.* **1989**, *34*, 531–562. [[CrossRef](#)]
35. Zvereva, E.L.; Paolucci, L.N.; Kozlov, M.V. Top-down factors contribute to differences in insect herbivory between saplings and mature trees in boreal and tropical forests. *Oecologia* **2020**, *193*, 167–176. [[CrossRef](#)] [[PubMed](#)]
36. Barton, K.E.; Koricheva, J. The ontogeny of plant defense and herbivory: Characterizing general patterns using meta-analysis. *Am. Nat.* **2010**, *175*, 481–493. [[CrossRef](#)] [[PubMed](#)]

37. Molleman, F.; Mandal, M.; Sokół-Łętowska, A.; Walczak, U.; Volf, M.; Mallick, S.; Moos, M.; Vodrážka, P.; Prinzing, A.; Mezzomo, P. Simulated herbivory affects the volatile emissions of oak saplings, while neighbourhood affects flavan-3-ols content of their leaves. *J. Chem. Ecol.* **2024**, *50*, 250–261. [CrossRef] [PubMed]
38. Ghirardo, A.; Heller, W.; Fladung, M.; Schnitzler, J.P.; Schroeder, H. Function of defensive volatiles in pedunculate oak (*Quercus robur*) is tricked by the moth *Tortrix viridana*. *Plant Cell Environ.* **2012**, *35*, 2192–2207. [CrossRef]
39. Pearse, I.S.; Gee, W.S.; Beck, J.J. Headspace volatiles from 52 oak species advertise induction, species Identity, and evolution, but not defense. *J. Chem. Ecol.* **2013**, *39*, 90–100. [CrossRef] [PubMed]
40. Jiang, Y.; Veromann-Jürgenson, L.L.; Ye, J.; Niinemets, Ü. Oak gall wasp infections of *Quercus robur* leaves lead to profound modifications in foliage photosynthetic and volatile emission characteristics. *Plant Cell Environ.* **2018**, *41*, 160–175. [CrossRef] [PubMed]
41. Klimm, F.S.; Weinhold, A.; Volf, M. Volatile production differs between oak leaves infested by leaf-miner *Phyllonorycter harrisella* (Lepidoptera: Gracillariidae) and galler *Neuroterus quercusbaccarum* (Hymenoptera: Cynipidae). *Eur. J. Entomol.* **2020**, *117*, 101–109. [CrossRef]
42. Copolovici, L.; Pag, A.; Kännaste, A.; Bodescu, A.; Tomescu, D.; Copolovici, D.; Soran, M.-L.; Niinemets, Ü. Disproportionate photosynthetic decline and inverse relationship between constitutive and induced volatile emissions upon feeding of *Quercus robur* leaves by large larvae of gypsy moth (*Lymantria dispar*). *Environ. Exp. Bot.* **2017**, *138*, 184–192. [CrossRef]
43. Reinhardt, A.; Kreuzwieser, J.; Thies, C.; Arnold, A. Variation of VOC emissions of oak trees infested by oak processionary moth. *Ann. For. Res.* **2023**, *66*, 4044–4056.
44. Volf, M.; Weinhold, A.; Seifert, C.L.; Holicová, T.; Uthe, H.; Alander, E.; Richter, R.; Salminen, J.-P.; Wirth, C.; van Dam, N.M. Branch-localized induction promotes efficacy of volatile defences and herbivore predation in trees. *J. Chem. Ecol.* **2021**, *47*, 99–111. [CrossRef] [PubMed]
45. Mrazova, A.; Houska Tahadlová, M.; Řehová, V.; Sam, K. The specificity of induced chemical defence of two oak species affects differently arthropod herbivores and arthropod and bird predation. *Arthropod-Plant Interact.* **2023**, *17*, 141–155. [CrossRef]
46. Jurkšienė, G.; Baranov, O.Y.; Kagan, D.I.; Kovalevič-Razumova, O.A.; Baliuckas, V. Genetic diversity and differentiation of pedunculate (*Quercus robur*) and sessile (*Q. petraea*) oaks. *J. For. Res.* **2020**, *31*, 2445–2452. [CrossRef]
47. Gossner, M.M.; Brandle, M.; Brandl, R.; Bail, J.; Muller, J.; Opgenoorth, L. Where is the extended phenotype in the wild? The community composition of arthropods on mature oak trees does not depend on the oak genotype. *PLoS ONE* **2015**, *10*, e0115733. [CrossRef] [PubMed]
48. Forest_Data_Bank. Forests on Maps. Available online: <https://www.bdl.lasy.gov.pl/portal/mapy-en> (accessed on 1 October 2018).
49. Kallenbach, M.; Oh, Y.; Eilers, E.J.; Veit, D.; Baldwin, I.T.; Schuman, M.C. A robust, simple, high-throughput technique for time-resolved plant volatile analysis in field experiments. *Plant J.* **2014**, *78*, 1060–1072. [CrossRef] [PubMed]
50. Volf, M.; Volfová, T.; Seifert, C.L.; Ludwig, A.; Engelmann, R.A.; Jorge, L.R.; Richter, R.; Schedl, A.; Weinhold, A.; Wirth, C. A mosaic of induced and non-induced branches promotes variation in leaf traits, predation and insect herbivore assemblages in canopy trees. *Ecol. Lett.* **2022**, *25*, 729–739. [CrossRef]
51. Southwood, R.T.E.; Wint, G.R.; Kennedy, C.E.J.; Greenwood, S.R. The composition of the arthropod fauna of the canopies of some species of oak (*Quercus*). *Eur. J. Entomol.* **2005**, *102*, 65–72. [CrossRef]
52. Molleman, F.; Walczak, U.; Melosik, I.; Baraniak, E.; Piosik, Ł.; Prinzing, A. What drives caterpillar guilds on a tree: Enemy pressure, leaf or tree growth, genetic traits, or phylogenetic neighbourhood? *Insects* **2022**, *13*, 367. [CrossRef] [PubMed]
53. Elbrecht, V.; Peinert, B.; Leese, F. Sorting things out: Assessing effects of unequal specimen biomass on DNA metabarcoding. *Ecol. Evol.* **2017**, *7*, 6918–6926. [CrossRef] [PubMed]
54. Molleman, F.; Walczak, U. Winter moth populations are isolated on co-occurring tree species with contrasting budburst-phenology. *Ecol. Entomol.* **2024**, *49*, 744–747. [CrossRef]
55. Uhler, J.; Redlich, S.; Zhang, J.; Hothorn, T.; Tobisch, C.; Ewald, J.; Thorn, S.; Seibold, S.; Mitesser, O.; Morinière, J. Relationship of insect biomass and richness with land use along a climate gradient. *Nat. Commun.* **2021**, *12*, 5946. [CrossRef] [PubMed]
56. Leray, M.; Yang, J.Y.; Meyer, C.P.; Mills, S.C.; Agudelo, N.; Ranwez, V.; Boehm, J.T.; Machida, R.J. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Front. Zool.* **2013**, *10*, 34. [CrossRef]
57. Ratnasingham, S.; Hebert, P.D. BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Mol. Ecol. Notes* **2007**, *7*, 355–364. [CrossRef] [PubMed]
58. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2016.
59. Oksanen, J.; Kindt, R.; Legendre, P.; O'Hara, B.; Stevens, M.H.H.; Oksanen, M.J.; Suggests, M. The vegan package. *Community Ecol. Package* **2007**, *10*, 719.
60. R_Core_Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2024.
61. Lämke, J.S.; Unsicker, S.B. Phytochemical variation in treetops: Causes and consequences for tree-insect herbivore interactions. *Oecologia* **2018**, *187*, 377–388. [CrossRef] [PubMed]
62. Bertić, M.; Schroeder, H.; Kersten, B.; Fladung, M.; Orgel, F.; Buegger, F.; Schnitzler, J.-P.; Ghirardo, A. European oak chemical diversity—From ecotypes to herbivore resistance. *New Phytol.* **2021**, *232*, 818–834. [CrossRef] [PubMed]
63. Pihain, M.; Gerhold, P.; Ducouso, A.; Prinzing, A. Evolutionary response to coexistence with close relatives: Increased resistance against specialist herbivores without cost for climatic-stress resistance. *Ecol. Lett.* **2019**, *22*, 1285–1296. [CrossRef]

64. Loreto, F.; Schnitzler, J.-P. Abiotic stresses and induced BVOCs. *Trends Plant Sci.* **2010**, *15*, 154–166. [[CrossRef](#)]
65. McCormick, A.C. Can plant–natural enemy communication withstand disruption by biotic and abiotic factors? *Ecol. Evol.* **2016**, *6*, 8569–8582. [[CrossRef](#)] [[PubMed](#)]
66. Becker, C.; Desneux, N.; Monticelli, L.; Fernandez, X.; Michel, T.; Lavoie, A.-V. Effects of abiotic factors on HIPV-mediated interactions between plants and parasitoids. *BioMed Res. Int.* **2015**, *2015*, 342982. [[CrossRef](#)] [[PubMed](#)]
67. Turlings, T.C.; Erb, M. Tritrophic interactions mediated by herbivore-induced plant volatiles: Mechanisms, ecological relevance, and application potential. *Annu. Rev. Entomol.* **2018**, *63*, 433–452. [[CrossRef](#)] [[PubMed](#)]
68. Lill, J.; Marquis, R.; Ricklefs, R. Host plants influence parasitism of forest caterpillars. *Nature* **2002**, *417*, 170–173. [[CrossRef](#)] [[PubMed](#)]
69. Wist, T.J.; Greis, R.; Evenden, M.L. Differential parasitism by a generalist parasitoid is mediated by volatile organic chemicals of the herbivore’s host. *Arthropod-Plant Interact.* **2015**, *9*, 515–527. [[CrossRef](#)]
70. Danner, H.; Desurmont, G.A.; Cristescu, S.M.; van Dam, N.M. Herbivore-induced plant volatiles accurately predict history of coexistence, diet breadth, and feeding mode of herbivores. *New Phytol.* **2018**, *220*, 726–738. [[CrossRef](#)] [[PubMed](#)]
71. Mezzomo, P.; Weinhold, A.; Aurová, K.; Jorge, L.R.; Kozel, P.; Michálek, J.; Nováková, N.; Seifert, C.L.; Volfová, T.; Engström, M. Leaf volatile and nonvolatile metabolites show different levels of specificity in response to herbivory. *Ecol. Evol.* **2023**, *13*, e10123. [[CrossRef](#)]
72. De Moraes, C.M.; Lewis, W.; Pare, P.; Alborn, H.; Tumlinson, J. Herbivore-infested plants selectively attract parasitoids. *Nature* **1998**, *393*, 570–573. [[CrossRef](#)]
73. McCormick, A.C.; Boeckler, G.A.; Köllner, T.G.; Gershenson, J.; Unsicker, S.B. The timing of herbivore-induced volatile emission in black poplar (*Populus nigra*) and the influence of herbivore age and identity affect the value of individual volatiles as cues for herbivore enemies. *BMC Plant Biol.* **2014**, *14*, 304.
74. Bruinsma, M.; Posthumus, M.A.; Mumm, R.; Mueller, M.J.; van Loon, J.J.; Dicke, M. Jasmonic acid-induced volatiles of *Brassica oleracea* attract parasitoids: Effects of time and dose, and comparison with induction by herbivores. *J. Exp. Bot.* **2009**, *60*, 2575–2587. [[CrossRef](#)] [[PubMed](#)]
75. Couchoux, C.; Seppä, P.; Van Nouhuys, S. Strong dispersal in a parasitoid wasp overwhelms habitat fragmentation and host population dynamics. *Mol. Ecol.* **2016**, *25*, 3344–3355. [[CrossRef](#)]
76. Hughes, J.S.; Cobbold, C.A.; Haynes, K.; Dwyer, G. Effects of forest spatial structure on insect outbreaks: Insights from a host-parasitoid model. *Am. Nat.* **2015**, *185*, E130–E152. [[CrossRef](#)]
77. Fraser, S.E.; Dytham, C.; Mayhew, P.J.; Mouillot, D.; Anderson, B.J. Community structure in ichneumonid parasitoids at different spatial scales. *Oecologia* **2008**, *157*, 521–530. [[CrossRef](#)] [[PubMed](#)]
78. Fraser, S.E.M.; Dytham, C.; Mayhew, P.J. The effectiveness and optimal use of Malaise traps for monitoring parasitoid wasps. *Insect Conserv. Divers.* **2008**, *1*, 22–31. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.