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Changes in volatile organic compounds (VOCs) content in resistant and non-resistant forest trees in response to bark beetle attack

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Abstract: The response of forest trees to bark beetle attack involves substantial changes in terpene content, which varies between resistant and non-resistant species. Terpenes serve as crucial chemical defences against pests, and their production can be influenced by prior attack history, water stress, and biotic interactions. Moreover, the effectiveness of terpenes in resisting bark beetle attack is influenced by their chemical composition. Different tree species produce different types and amounts of terpenes that can affect their overall resistance levels. In conifers, acyclic and cyclic monoterpenes and sesquiterpenes were significantly present in all samples. Acyclic monoterpenes, ketones, aldehydes, monocarboxylic acids and their esters, and aromatic and cyclic compounds have been identified in beech. A statistically significant decrease of compounds in infested trees was determined in pine (*cis*-β-ocimene, neo-allo-ocimene, terpinene-4-ol, and δ-cadinene), and fir (acetophenone, benzonitrile, phenol, and zonarene). In addition, increased benzaldehyde production was observed. However, in infested beech trees, only increased production of some aliphatic and aromatic compounds (2-butanone and 3,5-octadien-2-one, 2-methyl-4-pentenal and 2,4-hexadienal, octanoic acid, nonanoic acid, 3,4-dimethyl-2,5-furandione, acetophenone, benzeneacetaldehyde, 2-ethyl-1H-pyrrole, β-ionone-5,6-epoxide, β-cyclocitral, and geranyl acetone) was found. We investigated the changes in the terpene composition of surviving trees in bark beetle-infested stands of beech (*Fagus sylvatica*), pine (*Pinus sylvestris*), and fir (*Abies alba*). Our data showed that the distribution of different groups of volatile compounds varied according to the tree species.

Keywords: *Abies alba*; *Fagus sylvatica*; monoterpenes; *Pinus sylvestris*; sesquiterpenes

The first purpose of volatile organic compounds (VOCs) in plants was likely related to basic physiological and ecological interactions with their environment. Early in plant evolution, VOCs primarily served as byproducts of metabolic processes and adaptations to abiotic stressors, such as temperature fluctuations, high light intensity, or oxidative stress (Picazo-Aragonés et al. 2020). Over time, these compounds evolved to play more specialised roles in biotic interactions. For example, VOCs began mediating communication between plants and other organisms, such as attracting pollinators and seed dispersers or deterring herbivores and pathogens (Vivaldo et al. 2017). Co-evolutionary interactions between plants and insects have led to the formation of numerous secondary metabolites with complex modes of action. These compounds, such as terpenoids, benzenoids, phenylpropanoids, and fatty acid derivatives, balance attraction and repellence to optimise plant reproduction by attracting the most efficient pollinators (El-Sayed et al. 2018; Bouwmeester et al 2019). Another important function of these substances is to defend against insect and pathogen attack (Berryman 1972; Franceschi et al. 2005; Kersten et al. 2013). Trees use constitutive defences, including oleoresin secretion (a mixture of terpenes, sesquiterpenes, and diterpene acids), cell lignification, calcium oxalate crystal production, and inductive defences that are activated when the tree is attacked (Luchi et al. 2005; Krokene 2015). When traumatic resin channels are formed, resin flux and terpene production increase, polyphenolic cells in the cortex swell and proliferate, and the polyphenolic concentrations change (Franceschi et al. 2000; Krokene et al. 2003; Lieutier et al. 2003; Zeneli et al. 2006).

Volatile compounds in trees can change substantially in the presence of bark beetles (Coleoptera, Curculionidae, and Scolytinae). As highlighted in various studies (Franceschi et al. 2005; Krokene 2015; Biedermann et al. 2019), conifers rely on a combination of preformed defences, such as terpenoid resins, and inducible responses, such as traumatic resin ducts, to combat threats. For example, *Picea abies* produces mainly monoterpenes such as α- and β-pinene, camphene, sabinene, and sesquiterpenes, that is, β-caryophyllene, which are the main compounds in its resin (Jaakkola et al. 2023). Studies on Norway spruce trees infested by the European spruce bark beetle (*Ips typographus*) showed that biogenic volatile organic compound (BVOC) emissions increased considerably in infested trees compared with healthy ones, with emission rates being 6–20 times higher from infested bark than from constitutive needle emissions, leading to a substantial overall increase in BVOC emission rates (Giunta et al. 2016; Kolmašová et al. 2022; Jaakkola et al. 2023). This change in volatile compounds can affect atmospheric processes, potentially affecting the formation of secondary organic aerosols and influencing climatic feedback mechanisms (Monson et al. 1992; Esposito et al. 2016; Jakoby et al. 2019).

In deciduous trees, volatile compounds are attractive to many species of the genus *Scolytus*. In the case of *S. intricatus*, whose main host tree is oak (*Quercus* spp.), (E)-2-hexenal and hexanal were predominant BVOC in infested oak bark, in the buds, and in leaves and flowers (E,E)-α-farnesene (Vrkočová et al. 2000). Analysis of volatiles from the leaves of four non-host tree species of *Ips typographus*, i.e. *Betula pendula*, *B. pubescens*, *Populus tremula,* and *Sambucus nigra*, revealed three main classes of compounds, aliphatic (Z)-3-hexenyl acetate and (Z)-3-hexan-1-ol (mainly in *S. nigra* leaves), and monoterpenes and sesquiterpenes (e.g. α- and β-pinene, sabinene, β-caryophyllene, (E)-β-ocimene) were predominant in *B. pubescens* and *P. tremula* (Zhang et al. 1999).

Bark beetles such as *Ips typographus* and *Ips amitinus* showed a preference for conifers over deciduous trees (Wermelinger 2004). Studies have indicated that *Ips typographus* primarily targets Norway spruce trees, especially when they are weakened by drought or other stressors, because of volatile emissions that provide cues on tree vitality and suitability for attacks (Lehmanski et al. 2023). Similarly, *Ips amitinus* had a higher attack density and reproductive success on Norway spruce than on Scots pine, making it one of the most common bark beetle species in coniferous forests (Cocos et al. 2023). Additionally, studies on *Polygraphus proximus* have shown a preference for attacking *Abies* species over deciduous trees, indicating a trend of bark beetles favouring coniferous hosts across different species and geographic ranges (Takagi 2022).

Moreover, some phloem-feeding bark beetles (phytophagous) are vectors of phytopathogenic fungi, which, in some cases, contribute to tree death. The extent to which fungi benefit the beetles is complex. For example, volatile organic compounds (VOCs) emitted by fungi can act as pheromones or allelochemicals, thereby facilitating the association between beetles and fungi (Morath 2012). Changes in VOCs can also influence the behaviour of bark beetles. For instance, immature adults of the Eurasian spruce bark beetle (*I. typographus*) are attracted to food sources colonised by their fungal symbionts, but not saprophytic fungi, and this attraction is mediated by VOCs (Kandasamy et al. 2019). Notably, *Grosmannia penicillata* and other fungal symbionts can alter the volatile profile of spruce bark by converting the major monoterpenes into a mixture of oxidation derivatives attractive to bark beetles. Bornyl acetate was metabolised to camphor and α- and β-pinene to *trans*-4-thujanol and other oxidation products (Kandasamy et al. 2023). In summary, the presence of bark beetles can substantially alter volatile compounds in trees by introducing fungal symbionts that emit specific VOCs. These VOCs play a crucial role in facilitating the association between beetles and fungi, influencing beetle behaviour and potentially impacting beetle fitness. Understanding the relationships among bark beetles, terpene production, and tree resistance is crucial for developing effective management strategies to mitigate the effects of bark beetles on forest health and resilience.

MATERIAL AND METHODS

Study area. The experimental plots were located in the Czech Republic at six locations, with two plots for each tree species. Locations for beech (*Fagus sylvatica*) were Buková hora (50°40'36"N, 14°13'5"E), and Vrabinec (50°42'47"N, 14°13'6"E), for pine (*Pinus sylvestris*) Stará Boleslav (50°12'41"N, 14°42'24"E), and Podbrahy (50°13'33"N, 14°44'30"E), and for fir (*Abies alba*) Rychnov nad Kněžnou (50°9'25"N, 16°20'0"E), and Nové Město nad Metují (50°20'53"N, 16°9'54"E). More detailed information on the study site is provided in a previous paper (Soudek et al. 2024).

The selected stands comprised 10 control (resistant) and 15 bark-beetle-stressed (infested) trees. Resistant trees were free of bark beetle holes and trees with an approximate density of 0.15–0.2 borers per dm^2 were selected for the infested group. *Taphrorychus bicolor* was observed on beech, *Phaenops cyanea* was predominant on pine, and *Pityokteines spinidens* on firs.

Plant material sampling. Plant samples were collected from freshly cut needles and foliage in May 2022. Tree-canopy sampling was conducted using a tree plucker. Approximately 10 g of leaf tissue was collected from three locations on the crown and a composite sample was created to represent the test tree. Samples were collected from the lower green parts of the tree crowns. The height of the tree crown deployment (lower part) depended on the individual position of the tree and the tree species in the forest stand (4–21 m) and was therefore variable. Sampling was primarily conducted from the southern sunlit side of the tree to ensure the uniformity of the collection methodology. The collected plant material was immediately preserved in liquid nitrogen at the site and subsequently stored at −80 °C for further processing.

VOCs analysis. Approximately 10 mg [fresh weight (*FW*)] of pine or fir needles were weighed. The needles were not cut to avoid the non-standard release of green leaf volatiles (GLVs). Discs of 1 cm in diameter were cut from beech leaves, and the sample weight was set at approximately 30 mg (3–6 discs) because of the lower volatile emissions. Samples with 2 μL of internal standard mixture (7-XVOCs 1 ppm in pentane), giving 2 ng of each individual compound [1,2-dichloroethane; chlorobenzene; bromobenzene; 1-bromo-4-methoxybenzene (4-bromoanisole); 1,2-dibromobenzene; 1,6-dibromohexane; 1-bromo-3,5-dimethoxybenzene] in 20 mL tightly sealed screw-neck headspace vials (Gerstel GmbH & Co. KG, Germany) were analysed using a comprehensive two-dimensional gas chromatograph-mass spectrometer (LECO Pegasus 4D GC×GC-TOFMS, Leco Corporation, USA) equipped with an Agilent 7890 gas chromatograph (Agilent Technologies, USA) with a LECO quad-jet dual-stage thermal modulator. The system was equipped with a Gerstel MultiPurpose Sampler (Gerstel GmbH & Co. KG, Germany). The volatiles were released from the sample using the dynamic headspace technique in the Gerstel Dynamic Headspace Station (Gerstel, Germany), sorbed onto preconditioned Tenax TA-filled tubes (Gerstel, Germany), desorbed in a Thermal Desorption Unit (Gerstel, Germany), and transferred to the gas chromatograph columns via a temperature-programmable CIS4 inlet (Gerstel, Germany). The gas chromatograph was equipped as follows: precolumn (Phenomenex ZB 624plus, 1.3 m × 0.25 mm I.D. × 1.4 μm film, Phenomenex, USA); primary and modulatory columns (SGE Analytical Science BPX-5, 29. 3 + 0.1 m × 0.25 mm I.D. × 0.5 μm film, Trajan Scientific and Medical, Australia); secondary column and transfer line (SGE Analytical Science BPX-50, 1.9 + 0.21 m × 0.1 mm I.D. × 0.1 μm film). Built-in Purifier technology 5.7 purity helium (Air Products, Czech Republic) was used as carrier gas throughout the process from dynamic headspace to analysis. Mass spectra [electron ionisation (EI), –70 eV] were measured at a frequency of 100 Hz in the range of 14–600 amu.

Chromatograms were processed using the ChromaTOF software (Version 4.72.0.0, 2017). After optimisation of parameters [peak widths, signal-to-noise (S : N) ratio, etc.], delineation of chromatogram regions of interest, and exclusion of regions where background analytes (column bleed, siloxanes, etc.) were present, all chromatograms were processed at S : N = 100, exported with the exclusion of low ions (14–29 amu) problematic for analyte identification, re-imported, and processed again using the same method. Peak tables were exported, and simultaneously, a method for the calculation of retention indices (based on cochromatography of the C6–C40 aliphatic hydrocarbon mixture sample) was applied. Microsoft Excel and Microsoft Power Query (Version 2504, 2021) were used to filter the data and reduce the number of features to a reasonable level. For each experiment (fir, pine, and beech), a group of the highest peaks with high values of agreement between their mass spectra and the spectra from the libraries was created. These features, the groups of quantification ions, were revised and adapted for each and used to generate reference files, which were then implemented in the processing method. This method was used to reprocess all the chromatograms of the corresponding tree, resulting in mutually comparable results that were further used for statistical comparison. The tentative identification of analytes was based on a comparison of their spectra with those of mass libraries (NIST, LECO/Fiehn Metabolomics Library, and own user libraries) and published retention indices.

Statistical evaluation. First, the levels measured for each VOC were adjusted to determine the effects of the locality. This was done by dividing each value by the mean of the appropriate locality (calculated as the average of the infested tree mean for that locality and the uninfested tree mean for that locality) and multiplying the result by the mean compound level of the whole species. The resulting adjusted values were compared between the infected and resistant trees using the Mann-Whitney *U* test. The similarity or variability of the overall VOC profiles for each species was analysed by plotting the first two components after the principal component analysis (PCA). Only compounds detected in at least half of the samples were included in the PCA, before which the distribution of all included VOCs was standardised to a zero mean and standard deviation of 1. All reported *P*-values were two-tailed, and the default level of statistical significance was set at $\alpha = 0.05$, at which the false discovery rate (*FDR*) estimated by the Benjamini-Hochberg method was approximately 22%. To achieve an overall *FDR* of 5%, the significance level was decreased to 0.0025. Data was processed and analysed using MATLAB (Version R2021a, 2021).

RESULTS AND DISCUSSION

Emission of VOCs by trees plays a fundamental role in host selection, aggregation, and pest acceptance. VOCs include terpenoids, phenolics, and compounds derived from fatty acids and proteins.

To examine the changes in the abundance of VOCs, we selected stands of trees that were significantly infested with pests. These were mainly *Pityokteines spinidens* on fir, *Phaenops cyanea* on pine, and *Taphrorychus bicolor* on beech. Uninfested trees from the same site were used for comparison. The collected samples were subsequently analysed in the laboratory. We were aware that the analysis of cut needles and leaves does not provide data comparable to field measurements; however, given the number of samples and ensuring comparable sampling conditions, we considered this method of analysis appropriate.

Our results showed that trees infested with pests had increased or decreased production of some VOCs. A total of 43 compounds were identified in samples from pine (*P. sylvestris*), 64 compounds in samples from fir (*A. alba*) and 48 compounds in samples from beech (*F. sylvatica*). The representation of the different groups of volatile compounds varied according to the tree species. Significant differences were observed, particularly between conifers (fir and pine) and beeches. In conifers, acyclic and cyclic monoterpenes and sesquiterpenes were significantly represented in the samples. In the case of beech, large amounts of alcohols, ketones, aldehydes, saturated and unsaturated monocarboxylic acids, and aromatic compounds were detected. A list of the identified compounds with median values for the infested and uninfested trees is provided in Tables 1–3.

Table 1. Content (expressed as median of peak area·mg^{-1} *FW*) of detected volatile organic compounds (VOCs) adjusted for the effect of locality in needles between uninfested and infested pine (*Pinus sylvestris*)

Compound	*P*	Median (peak area·mg^{-1})	
		uninfested (n_U = 20)	infested (n_I = 30)
Alcohols			
2-hexen-1-ol	0.6860	63 139 800	62 269 300
Aldehydes			
2-hexenal	0.6068	60 434 025	59 561 984
2,4-hexadienal	0.5060	11 922 445	11 897 224
Ketones			
(Z)-undec-6-en-2-one	0.2566	20 668	9 029
2-undecanone	0.2059	202 583	89 514
Aromatic compounds			
Methyl salicylate	**0.1985**	**71 044**	**12 764**
Acyclic monoterpenes			
β-myrcene	0.2791	15 876 742	18 422 635
Linalool	0.3280	274 911	169 717
trans,trans-alloocimene	0.2229	500 492	849 491
Neo-allo-ocimene	0.0460^{*}	813 436	1 418 587
cis-β-ocimene	0.0102^{*}	36 748 345	61 342 791
trans-β-ocimene	**$0.0952^{\dagger}$**	**1 409 689**	**1 972 718**
Monocyclic monoterpenes			
p-mentha-1,5-dien-8-ol	0.8074	290 786	292 149
α-phellandrene	0.7248	30 305 573	37 536 195
α-terpineol	0.7053	518 997	417 612
Terpinen-4-ol	0.0489^{*}	1 731 134	1 047 525
α-terpinyl acetate	0.4072	92 861	51 818
β-terpinyl acetate	0.4895	232 882	346 301
γ-terpinene	0.1878	4 635 131	5 993 383
Terpinolene	0.1792	2 651 943	3 889 323
p-cymenene	0.3823	3 937 530	2 827 034
m-cymenene	0.1967	1 200 658	1 585 121
m-cymen-8-ol	**0.2770**	**1 539 764**	**793 070**
p-cymen-8-ol	$0.0552^{\dagger}$	428 199	256 586
Limonene	**0.2941**	**33 525 469**	**53 083 482**
O-methylthymol	0.8859	331 274	216 209
Bicyclic monoterpenes			
(1*R*)-(+)-α-pinene	0.9568	7 972 479	8 016 422
(1*S*)-(−)-α-pinene	0.2937	18 369 109	25 057 396
(1*S*)-(−)-β-pinene	0.6669	5 719 057	7 526 017
Bornyl acetate	0.9066	3 169 514	3 302 287
(−)-camphene	0.3973	1 331 561	1 033 648
(+)-camphene	0.6386	865 808	1 003 140
(+)-2-carene	**0.2000**	**4 233 851**	**2 510 345**
(+)-3-carene	0.7842	52 611 043	63 407 888
Tricyclic monoterpenes			
Tricyclene	0.7041	131 575	135 353
Sesquiterpenes			
(−)-(E)-caryophyllene	0.1629	1 845 572	1 260 962
α-cadinene	$0.0586^{\dagger}$	442 348	221 670
δ-cadinene	0.0314^{*}	10 522 551	3 817 549
cis-calamenene	$0.0977^{\dagger}$	70 689	40 290
α-humulene	$0.0520^{\dagger}$	525 659	345 774
α-muurolene	$0.0639^{\dagger}$	1 676 084	741 670
γ-muurolene	$0.0586^{\dagger}$	643 293	274 973
Zonarene	$0.0828^{\dagger}$	323 772	153 578

†,*,**,***significant differences between treatments at $P < 0.1$, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; bold – compounds that were identified in less than 50% of samples from a single site and treatment; *FW* – fresh weight; n_I – number of infested trees (n_I = 30); n_U – number of uninfested trees (n_U = 20); samples were collected on two sites ($n_I : n_U$ = 15 : 10 per site)

In the case of pine, statistically significant differences were observed for four compounds (out of the 43 identified) at *P*-levels less than 5%. *Cis*-β-ocimene and neo-allo-ocimene were two acyclic monoterpenes, where a 40–74% increase in production was observed in infested trees. The other two were one cyclic monoterpene (terpinene-4-ol) and one sesquiterpene (δ-cadinene), where the production of these compounds decreased by 40% and 64%, respectively, in infested trees (Figure 1). Eight additional compounds were identified at a significance *P*-level of 10%: six sesquiterpenes and one monoterpene, whose production in infested trees decreased by 34–64%, and one acyclic monoterpene (*trans*-β-ocimene), where production increased by 40% (see Figures 2 and 3).

Our observed increase in monoterpene production is consistent with this function. Indeed, monoterpenes serve as host tree defences; however, their toxicity to pine beetles varies, with

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Table 2. Content (expressed as median of peak area·mg^{-1} *FW*) of detected volatile organic compounds (VOCs) adjusted for the effect of locality in needles between uninfested and infested fir (*Abies alba*)

Compound	P	Median (peak area·mg^{-1})	
		uninfested (n_U = 20)	infested (n_I = 30)
Alcohols			
3-hexen-1-ol	0.2233	26 662 031	20 254 071
Esters			
2-heptanol, acetate	0.4945	1 528 782	1 010 932
2,6-dimethyl-1,6-heptadien-4-ol acetate	**0.7867**	**73 533**	**65 953**
Aldehydes			
Hexanal	0.6703	874 495	769 212
2-hexenal	0.7141	38 698 636	35 447 581
2-methyl-4-pentenal	0.2632	2 361 307	2 589 473
Ketones			
6-methyl-6-hepten-2-one	0.6947	453 898	509 306
2-nonanone	**0.5538**	**1 624 349**	**1 198 519**
Aromatic compounds			
Acetophenone	0.0008^{***}	448 814	795 642
Benzaldehyde	$0.0904^{\dagger}$	1 761 417	2 599 544
Benzonitrile	0.0120^{*}	108 228	183 652
Mesitylene	0.4663	333 422	307 164
Methyl salicylate	0.8762	22 098	21 340
Phenol	0.0029^{**}	298 484	549 895
Acyclic monoterpenes			
β-myrcene	0.4663	31 320 963	28 997 039
cis-geraniol	0.2985	938 800	715 898
Geranyl acetate	0.2387	1 374 753	3 620 041
Linalool	0.7141	871 509	738 684
Citronellol acetate	0.5982	228 329	270 623
trans,trans-alloocimene	0.4059	644 697	520 385
trans-β-ocimene	**0.7180**	**4 115 892**	**5 355 828**
Monocyclic monoterpenes			
1,3,8-*p*-menthatriene	0.2340	155 788	122 637
α-phellandrene	0.9591	1 094 834	1 461 028
α-terpineol	0.8495	1 390 311	1 332 952
β-terpinyl acetate	0.8353	680 428	724 959
γ-terpinene	0.4625	1 234 758	1 558 004
Terpinolene	0.5450	1 954 925	1 968 206
p-cymene	0.1007	6 307 442	4 383 147
p-cymenene	$0.0656^{\dagger}$	2 833 865	2 393 359
Limonene	$0.0535^{\dagger}$	77 349 586	54 593 753
trans-dihydrocarvone	**0.9826**	**97 581**	**96 163**
Bicyclic monoterpenes			
(1*R*)-(+)-α-pinene	0.6008	11 481 620	13 746 040
(1*S*)-(−)-α-pinene	0.9043	31 714 488	27 010 351
(1*S*)-(−)-β-pinene	0.5725	21 798 558	32 102 879
2-bornene	0.8353	451 744	482 903
Borneol	0.6994	9 182 525	11 559 675
Bornyl acetate	0.1249	20 060 885	41 983 132
(−)-camphene	0.3565	36 152 903	26 792 692
(+)-camphene	0.2548	15 415 129	14 331 817
Eucalyptol	0.7989	27 434	34 969
Sabinene	**0.5409**	**43 210 836**	**29 906 636**
Tricyclic monoterpenes			
Tricyclene	$0.0653^{\dagger}$	2 230 218	1 411 878
Sesquiterpenes			
(−)-(E)-caryophyllene	0.6276	16 320 682	128 97 675
(+)-aromadendrene	0.4416	2 537 682	2 234 961
(E)-β-famesene	0.1773	91 258	136 621
α-fadinol	0.1201	291 915	198 229
τ-cadinol	$0.0670^{\dagger}$	600 560	408 107
δ-cadinene	$0.0731^{\dagger}$	20 825 370	13 671 224
α-calacorene	$0.0731^{\dagger}$	128 907	76 846
cis-calamenene	$0.0700^{\dagger}$	288 650	162 527
α-humulene	0.6740	12 047 818	6 819 201
α-muurolene	0.1749	6 211 257	4 161 597
a*R*-himachalene	0.9921	295 411	234 817
Himachala-2,4-diene	$0.0993^{\dagger}$	2 130 698	1 198 763
Himachalene-1,4-diene	0.3429	1 097 603	2 483 584
δ-selinene	0.5902	133 984	76 466
Zonarene	0.0488^{*}	1 640 902	978 969
α-amorphene	0.6857	1 981 264	1 117 952
β-bisabolene	0.8000	3 074 881	906 059
Cubebene	0.1965	1 245 221	548 861
Tricyclic sesquiterpenes			
β-copaene	0.2035	5 672 939	3 885 910
Clovene	0.8663	901 850	835 283
Longipinene	0.9583	4 091 909	3 882 507
Ylangene	**0.5373**	**134 331**	**123 378**

†,*,**,***significant differences between treatments at $P < 0.1$, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; bold – compounds that were identified in less than 50% of samples from a single site and treatment; *FW* – fresh weight; n_I – number of infested trees (n_I = 30); n_U – number of uninfested trees (n_U = 20); samples were collected on two sites ($n_I : n_U$ = 15 : 10 per site)

Table 3. Content (expressed as median peak area·mg^{-1} *FW*) of detected volatile organic compounds (VOCs) adjusted for the effect of locality in leaves between uninfested and infested beech (*Fagus sylvatica*)

Compound	P	Median (peak area·mg^{-1})	
		uninfested (n_U = 20)	infested (n_I = 30)
Acids, anhydrids and esters			
Hexanoic acid	0.0677†	58 861	184 234
Hexenoic acid	0.0655†	8 813	36 058
Nonanoic acid	0.0288*	45 290	103 444
Octanoic acid	0.0203*	55 625	103 951
3-hexenoic acid, ethyl ester	0.2067	107 853	76 040
3,4-dimethyl-2,5-furan-dione	0.0476*	3 577	10 264
Ketones			
2-butanone	0.0401*	164 208	340 544
Sulcatone	0.1642	450 496	1 033 080
3,5-octadien-2-one	0.0290*	66 843	219 613
4,6-dimethyloctane-3,5-dione	0.3488	2 151 558	3 443 056
1,4-cyclohex-2-enedione	0.1709	37 529	46 357
Aldehydes			
Hexanal	0.2743	3 018 053	3 366 833
Heptanal	0.1807	618 960	523 409
Nonanal	0.1881	1 815 746	1 848 731
Decanal	0.0609†	782 403	867 143
2-methyl-4-pentenal	0.0007***	1 703 540	2 317 126
2-methyl-undecanal	0.2743	35 225	47 100
2-hexenal	0.1470	27 472 764	32 593 104
2-heptenal	0.3457	94 429	208 412
2,4-hexadienal	0.0360*	1 737 350	4 805 648
(Z,Z)-2,4-heptadienal	0.1509	125 604	277 264
(E,E)-2,4-heptadienal	0.1291	46 530	185 977
4-oxohex-2-enal	**0.0990**†	**781 213**	**1 877 321**
Alcohols			
2-penten-1-ol	0.0590†	1 484 038	2 692 337
3-hexen-1-ol	0.4616	18 256 331	16 422 307
2-ethyl-1-hexanol	0.5734	156 643	161 304
2-methyl-4-hexen-3-ol	0.0641†	45 139	68 193
Alkane hydrocarbons			
Hexadecane	0.0609†	1 103 049	1 269 404
Aromatic compounds			
Acetophenone	0.0006***	89 480	146 281
Benzaldehyde	0.0943†	2 714 821	3 548 397
Benzeneacetaldehyde	0.0192*	387 476	877 865
Benzoic acid	0.5055	61 584	82 707
Benzyl alcohol	0.2958	1 518 467	2 395 117
Methyl salicylate	0.0765†	114 282	434 841
1-phenoxypropan-2-ol	0.0642†	132 548	216 451
Orcinol	0.1082	8 900	35 818
2-ethyl-1H-pyrrole	0.0192*	70 371	234 538
2-pentylfuran	0.5322	86 227	101 015
Phenylethyl alcohol	**0.2630**	**61 326**	**1 442 927**
Cyclic compounds			
α-ionone	0.6707	109 336	70 264
trans-β-ionone	0.0579†	70 508	125 140
β-ionon-5,6-epoxide	0.0290*	7 199	25 905
Isophorone	0.4666	35 926	62 945
1,4-*p*-menthadien-7-al	0.1082	69 363	107 086
2,3-epoxy-1-cyclohexanone	0.1821	15 083	41 523
β-cyclocitral	0.0216*	131 357	464 638
2-butyl tetrahydrofuran	0.2104	324 442	401 070
Acyclic monoterpenes			
Geranyl acetone	0.0163*	85 851	194 661

†,*,**,*** significant differences between treatments at $P < 0.1$, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; bold – compounds that were identified in less than 50% of samples from a single site and treatment; *FW* – fresh weight; n_I – number of infested trees (n_I = 30); n_U – number of uninfested trees (n_U = 20); samples were collected on two sites ($n_I : n_U$ = 15 : 10 per site)

(–)-limonene being the most toxic and (–)-α-pinene the least (Chiu et al. 2017). In contrast, bark beetles have evolved to use monoterpenes as host search cues and pheromone precursors (Chiu, Bohlmann 2022). For example, lodgepole pine (*P. banksiana*) has a higher content of α-pinene, a beetle pheromone precursor, but a lower content of defence compounds such as 3-carene compared to Scots pine (*Pinus contorta*) (Clark et al. 2014). Some studies have shown that *P. sylvestris* releases higher concentrations of volatile organic compounds, including monoterpenes, than *Picea abies* (Gabriel et al. 2015; Skulberg et al. 2019). These emissions may vary depending on the physiological

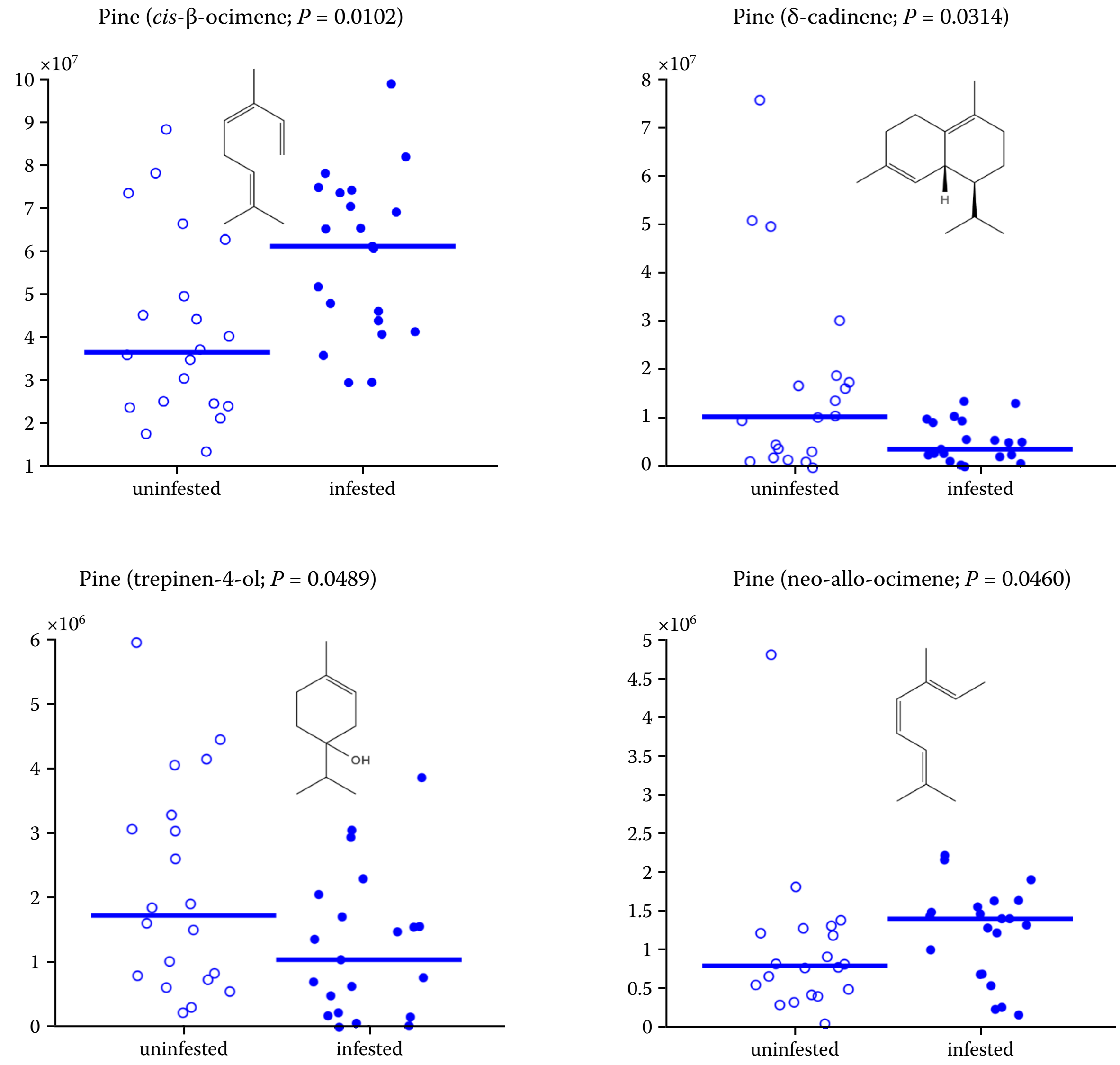

Figure 1. Selected volatile organic compounds with significantly different production ($P < 0.05$) in needles between uninfested and infested pine (*Pinus sylvestris*)

n_I – number of infested trees ($n_I = 30$); n_U – number of uninfested trees ($n_U = 20$); samples were collected on two sites ($n_I : n_U = 15 : 10$ per site); content (expressed as peak area) is adjusted for the effect of locality; significant differences between treatments for different compounds are in title of each figure

stage of the pine needles, with litter releasing more VOCs than the green needles (Santonja et al. 2019). Kovalchuk et al. (2015) also list monoterpenes and sesquiterpenes as the main VOCs emitted from the bark of pine (*Pinus silvestris*) after infestation with the beetle *Hylobius abietis*. For monoterpenes, an increase of approximately 20% was observed in VOCs production (mainly 3-carene and limonene). The production of sesquiterpenes (α-muurolene, δ-cadinene, *trans*-β-farnesene, longicyclene, longifolene, and *trans*-β-caryophyllene) increased up to 8-fold.

For fir, statistically significant differences were determined at *P*-levels below 5% for 4 compounds (out of the 64 identified). In this study, three phenolic compounds (acetophenone, benzonitrile, and phenol) increased production in infested trees by 70–84%, and one sesquiterpene (zonarene) decreased production in infested trees by 40% (Figure 4). At a 10% significance *P*-level, we identified eight additional compounds (five sesquiterpenes, four monoterpenes, and one phenolic compound). In the case of terpenes, a 16–44% reduction was observed in production in the infested trees, and for the phe-

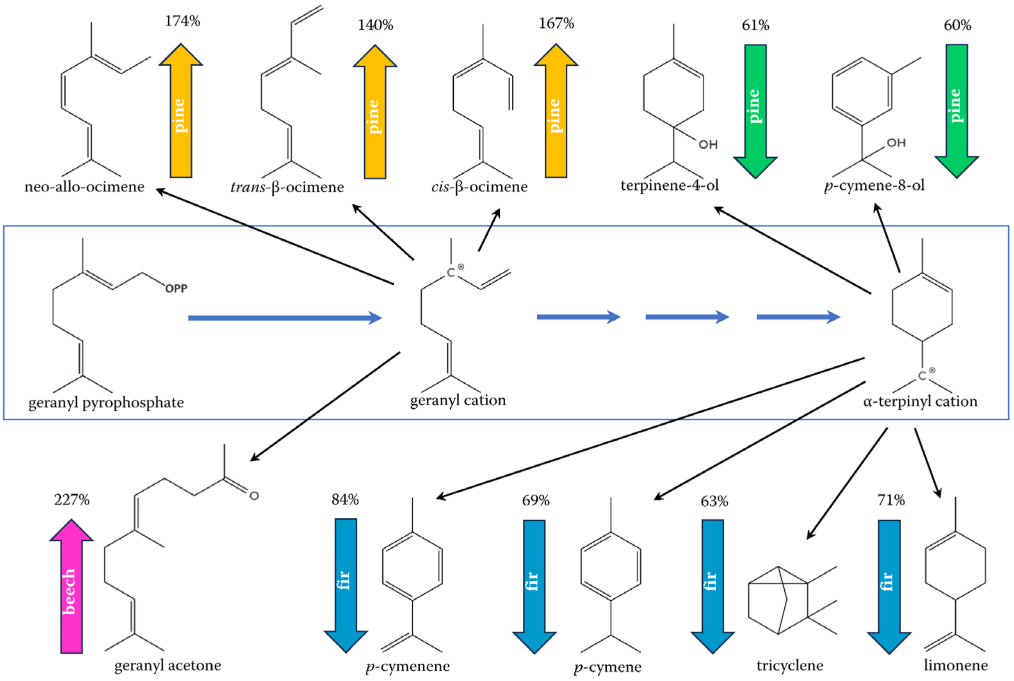

Figure 2. Increase or decrease of selected monoterpenes with significantly different production ($P < 0.1$) in leaves or needles between uninfested and infested trees: Pine (*Pinus silvestris*), fir (*Abies alba*), and beech (*Fagus sylvatica*)

Arrow pointing upwards – an increase in compound production in infested trees; arrow pointing downwards – a decrease in compound production in infested trees; percentages above the arrow – the percentage increase or decrease in production of the compound in infested trees (100% = uninfested trees)

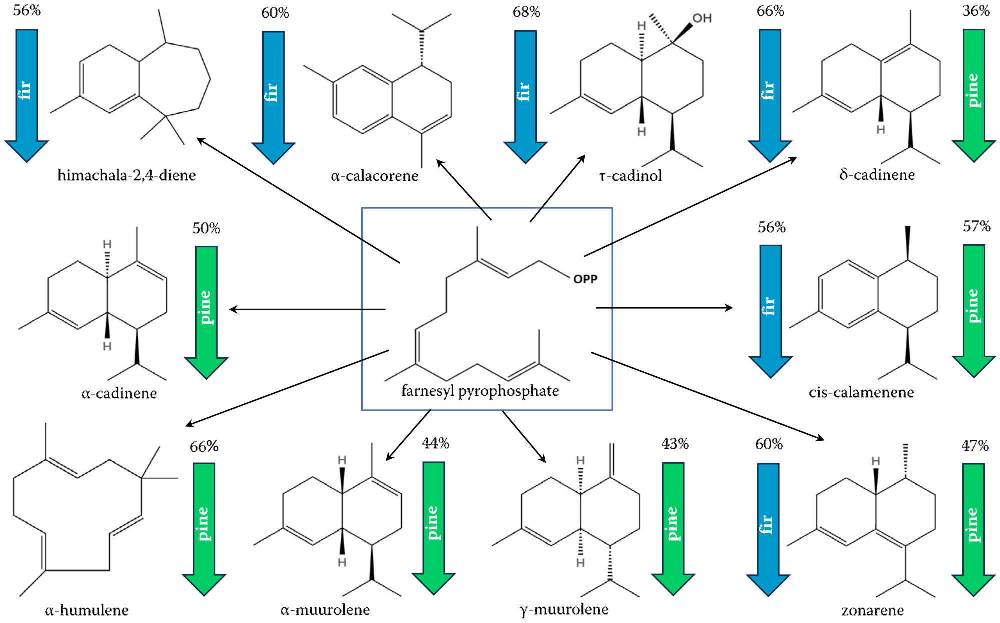

Figure 3. Increase or decrease of selected sesquiterpenes with significantly different production ($P < 0.1$) in needles between uninfested and infested trees [pine (*Pinus silvestris*) and fir (*Abies alba*)]

Arrow pointing upwards – an increase in compound production in infested trees; arrow pointing downwards – a decrease in compound production in infested trees; percentages above the arrow – the percentage increase or decrease in production of the compound in infested trees (100% = uninfested trees)

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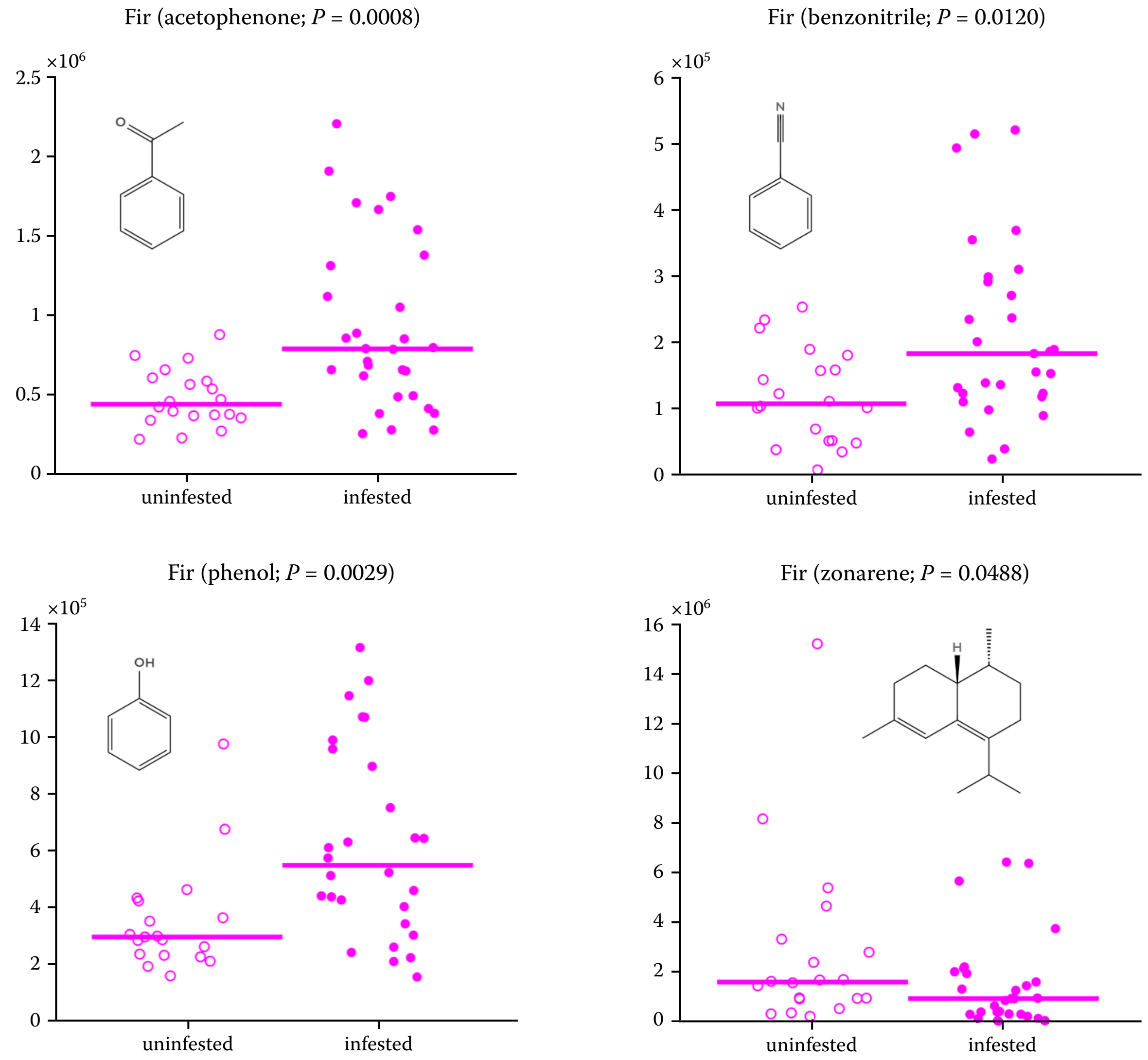

Figure 4. Selected volatile organic compounds with significantly different production ($P < 0.05$) in needles between uninfested and infested fir (*Abies alba*)

n_I – number of infested trees ($n_I = 30$); n_U – number of uninfested trees ($n_U = 20$); samples were collected on two sites ($n_I : n_U = 15 : 10$ per site); content (expressed as peak area) is adjusted for the effect of locality; significant differences between treatments for different compounds are in title of each figure

nolic compound (benzaldehyde), a 48% increase was observed in production (Figures 2, 3 and 5).

The increase in the production of phenolic compounds, such as acetophenone, may be related to the active defence reaction of the fir tree. Sullivan (2005) showed that acetophenone reduced the attractiveness of the male southern pine beetle *Dendroctonus frontalis*, especially at low release rates, and altered the sex ratio of captured beetles. Erbilgin et al. (2008) compared the effectiveness of acetophenone against the aggregation pheromone of the western pine beetle *Dendroctonus brevicomis*, and found that it was superior to verbenone in reducing attraction. In addition, Nones et al. (2022) reported that acetophenone levels increased in cork oak seedlings inoculated with ragweed-associated fungi, suggesting its role in mediating beetle behaviour in response to interactions with fungi. Collectively, these studies highlighted the ecological importance of acetophenone in beetle attraction and behaviour. In the case of monoterpenes, our analyses showed that their production decreased in infested trees, in contrast to that of pines. But, for example, Zamponi et al. (2007) reported that *Abies alba* resin showed changes in monoterpene composition in response

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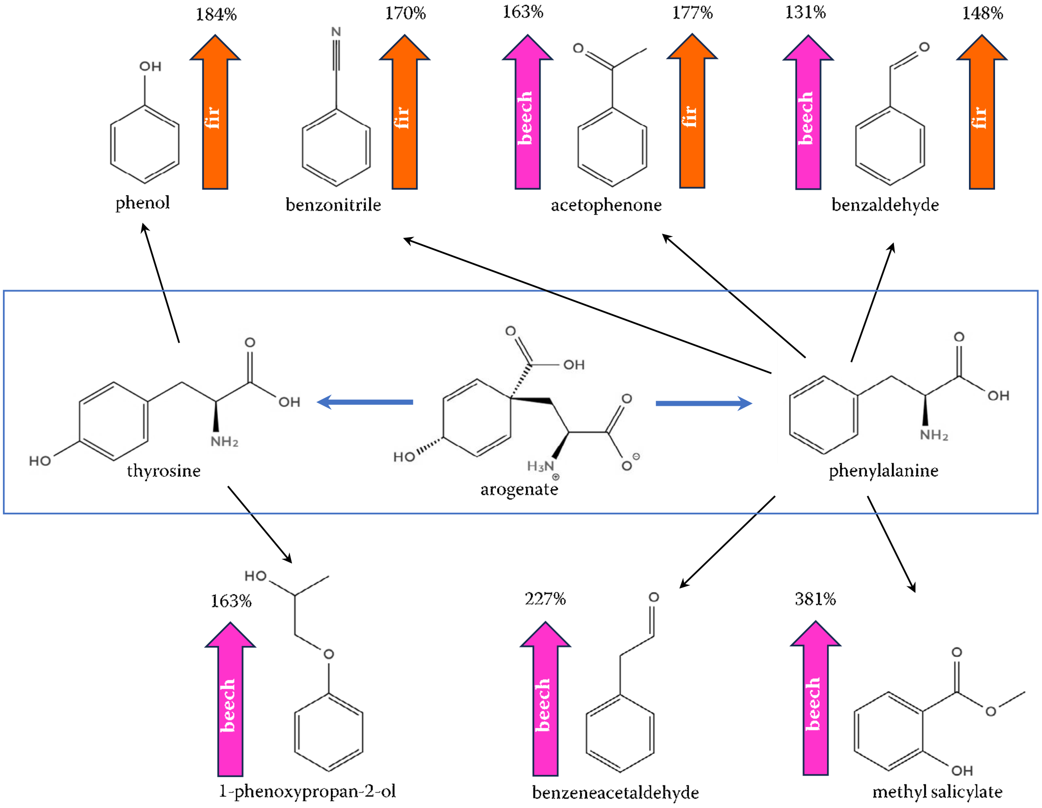

Figure 5. Increase or decrease of selected phenolic compounds with significantly different production ($P < 0.1$) in leaves or needles between uninfested and infested trees (fir (*Abies alba*) and beech (*Fagus sylvatica*))

Arrow pointing upwards – an increase in compound production in infested trees; arrow pointing downwards – a decrease in compound production in infested trees; percentages above the arrow – the percentage increase or decrease in production of the compound in infested trees (100% = uninfested trees)

to infection by *Heterobasidion* fungi, in particular an increase in (–)-α-pinene and (–)-camphene and a decrease in β-phellandrene. Our measurements were performed using needles, which may explain these differences.

The third tree species tested was beech, for which we identified 13 compounds (out of 48 identified) with statistically significant differences between the infested and uninfested trees at the 5% significance *P*-level. The identified compounds came from the groups of ketones (2-butanone and 3,5-octadien-2-one), aldehydes (2-methyl-4-pentenal and 2,4-hexadienal), monocarboxylic acids and their esters (octanoic acid, nonanoic acid, and 3, 4-dimethyl-2,5-furandione), aromatic (acetophenone, benzeneacetaldehyde, and 2-ethyl-1H-pyrrole) and cyclic compounds (β-ionone-5,6-epoxide and β-cyclocitral) and acyclic monoterpenes (geranyl acetone) (Figure 6). Eleven compounds were identified at the 10% significance *P*-level. All these compounds showed an increase in their production in infested trees in the range of 15–309% with the highest increase observed for hexenoic acid (Figures 2, 5, 7, and 8).

Tollsten and Müller (1996) reported that beech leaves mainly secrete sabinene and other isoprenoids, although at lower concentrations than coniferous trees, and that their emissions correlate with the ambient temperature. In the present study, only geranyl acetone was detected, followed by several aliphatic hydrocarbons. However, their production is primarily associated with fungus-infested beech wood, and they serve as potential semiochemicals for saproxylic insects and their natural enemies, mediating complex ecosystem interactions (Leather et al. 2014). Furthermore, as in the case of fir, we observed a significant increase in the production of acetophenone and other phenolic compounds. These factors may have a similar function in reducing attractiveness and influencing beetle behaviour. Other substances identified include *trans*-β-ionone, β-cyclocitral,

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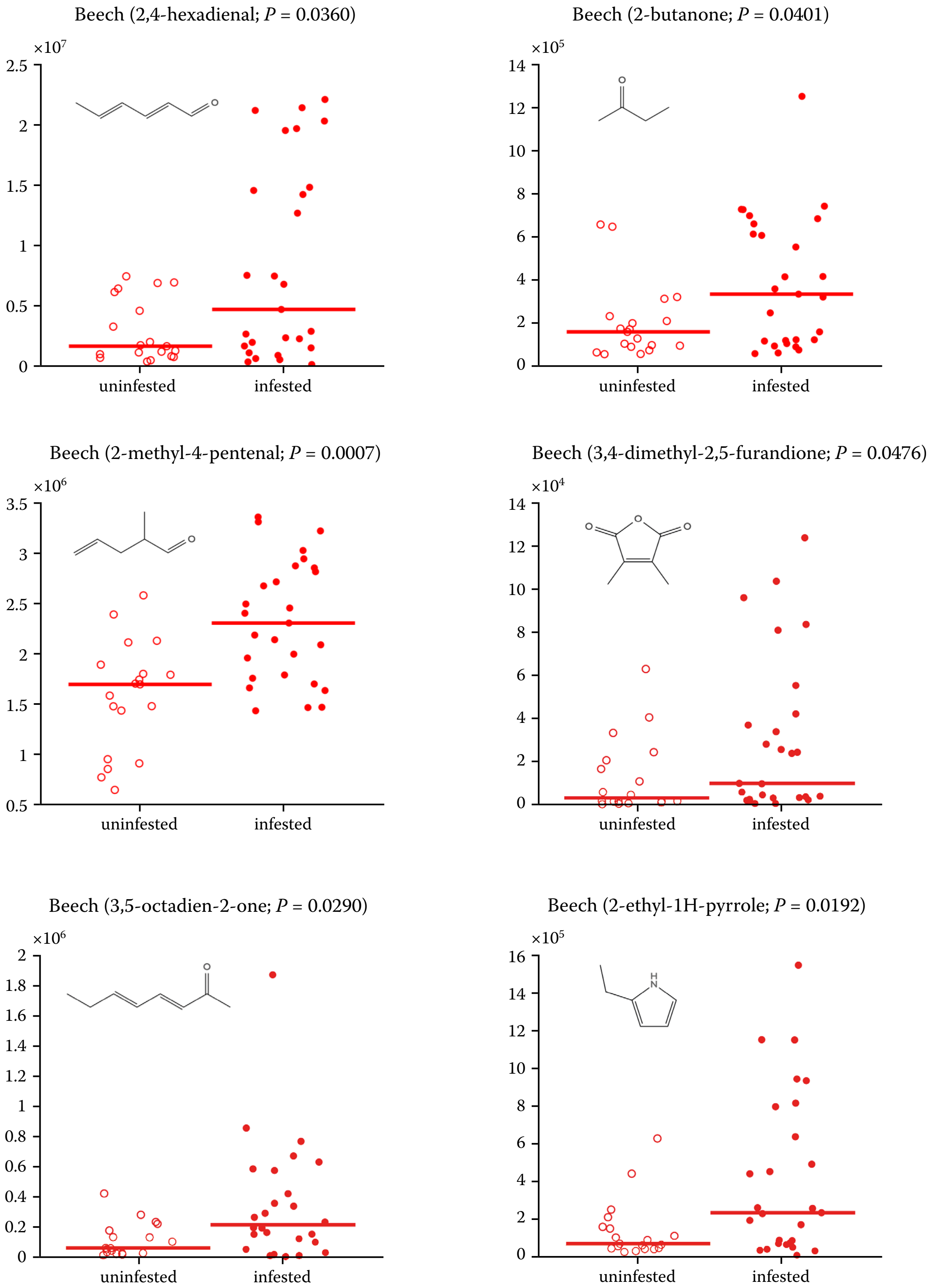

Figure 6. Selected volatile organic compounds with significantly different production ($P < 0.05$) in leaves between uninfested and infested beech (*Fagus sylvatica*)

n_I – number of infested trees ($n_I = 30$); n_U – number of uninfested trees ($n_U = 20$); samples were collected on two sites ($n_I : n_U = 15 : 10$ per site); content (expressed as peak area) is adjusted for the effect of locality; significant differences between treatments for different compounds are in title of each figure

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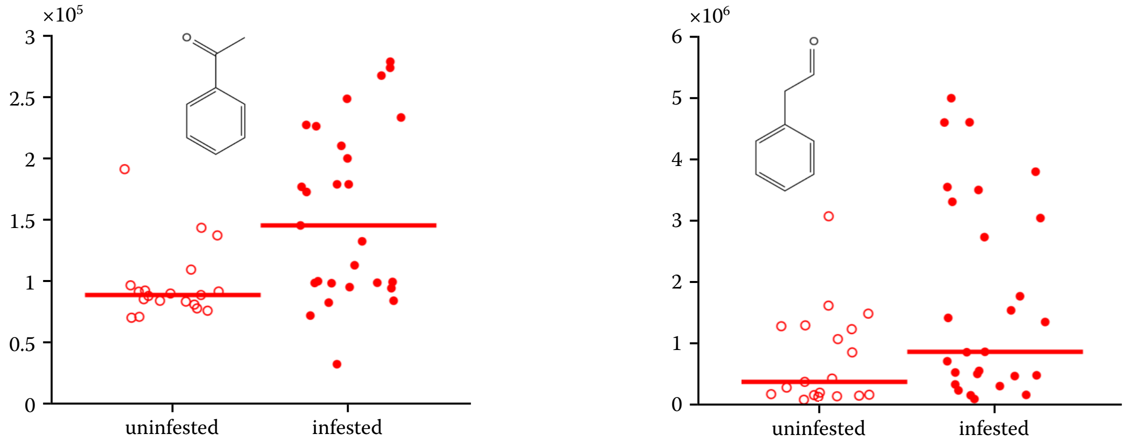
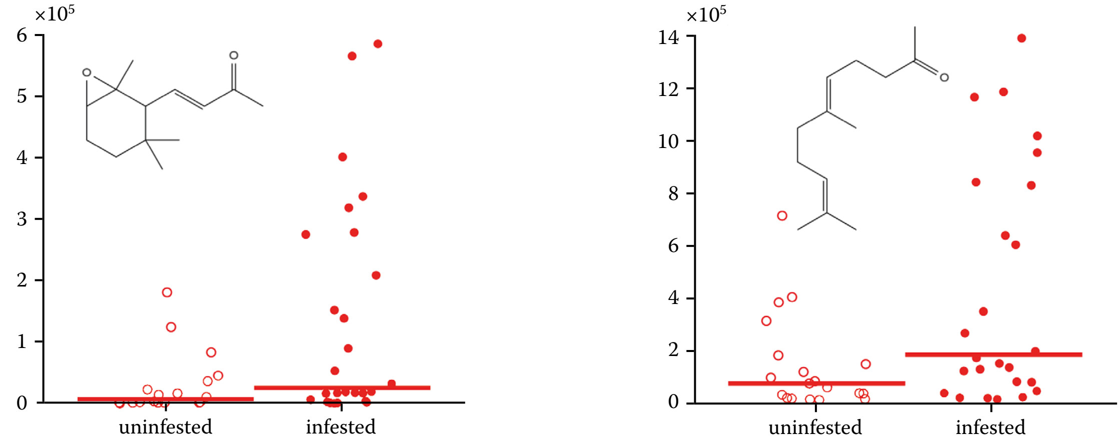
Beech (acetophenone; *P* = 0.0006)

Beech (benzeneacetaldehyde; *P* = 0.0192)



Beech (β-ionon-5,6-epoxide; *P* = 0.0290)

Beech (geranyl acetone; *P* = 0.0163)



Beech (nonanoic acid; *P* = 0.0288)

Beech (octanoic acid; *P* = 0.0203)

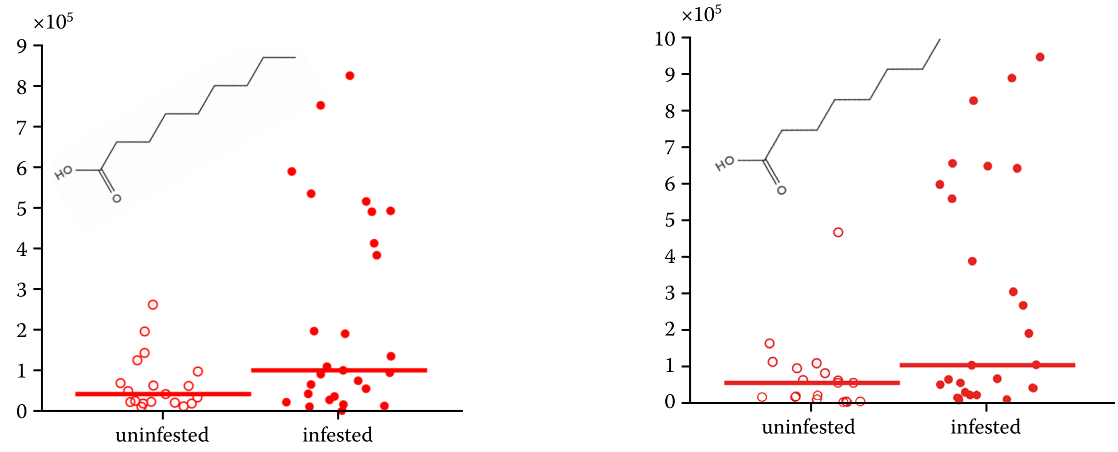


Figure 6. To be continued

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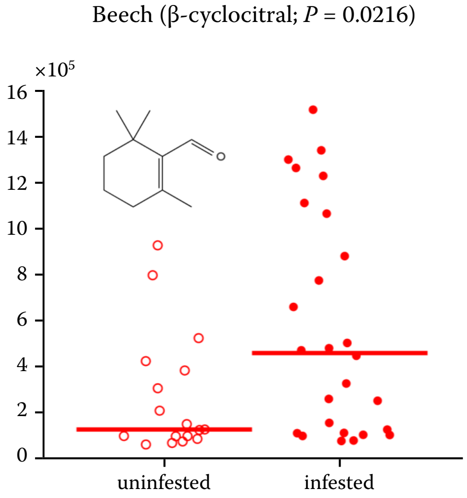

Figure 6. To be continued

and β-ionone-5,6-epoxide, which are products of specific oxidative cleavage of β-carotene (Paparella et al. 2021). These substances may act as both pest repellents and unwanted attractants for beeches. For example, in *Arabidopsis*, overexpression of AtCCD1 increases β-ionone emission, which reduces damage caused by the crucifer flea beetle (Wei et al. 2011). In contrast, similar volatile organic compounds from *Capsicum annuum* and *C. frutescens*, especially α-ionone and β-ionone, attract the *Lasioderma serricorne* beetle (Guarino et al. 2021).

Data from the PCA (Figure 9) indicated different species-specific production of volatiles. In pine, a more homogeneous group of individuals appeared to have been affected by the beetle, whereas in fir, both groups of trees (infested and uninfested) appeared to be equally heteroge-

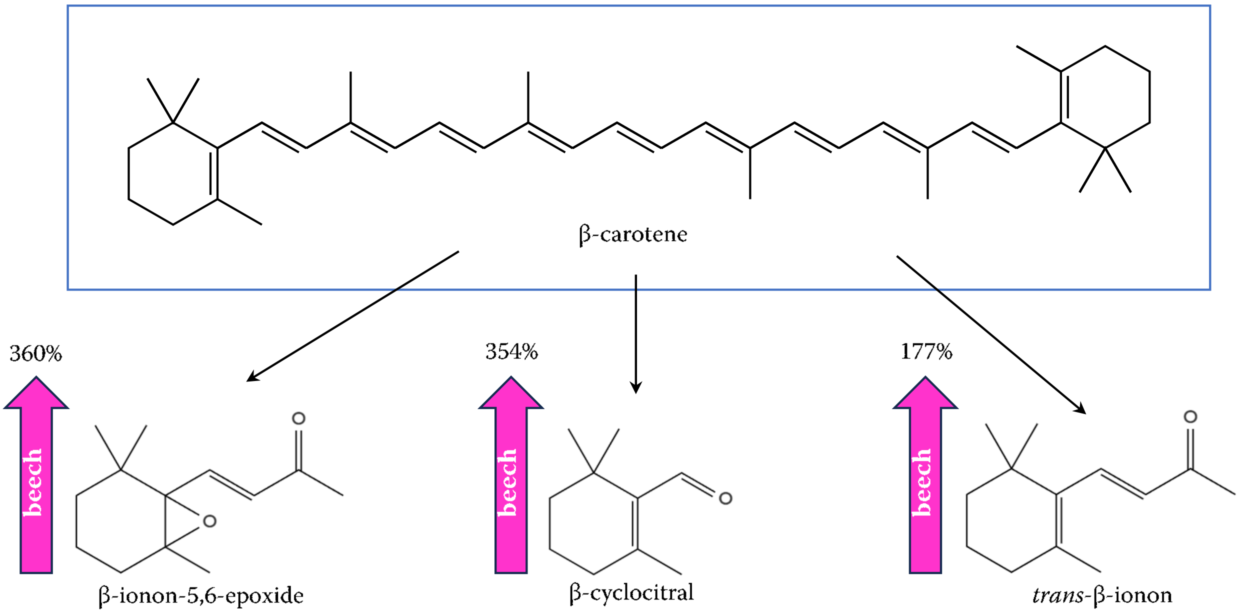

Figure 7. Increase or decrease of selected compounds associated with carotene catabolism with significantly different production ($P < 0.1$) in leaves between uninfested and infested trees [beech (*Fagus sylvatica*)]

Arrow pointing upwards – an increase in compound production in infested trees; arrow pointing downwards – a decrease in compound production in infested trees; percentages above the arrow – the percentage increase or decrease in production of the compound in infested trees (100% = uninfested trees)

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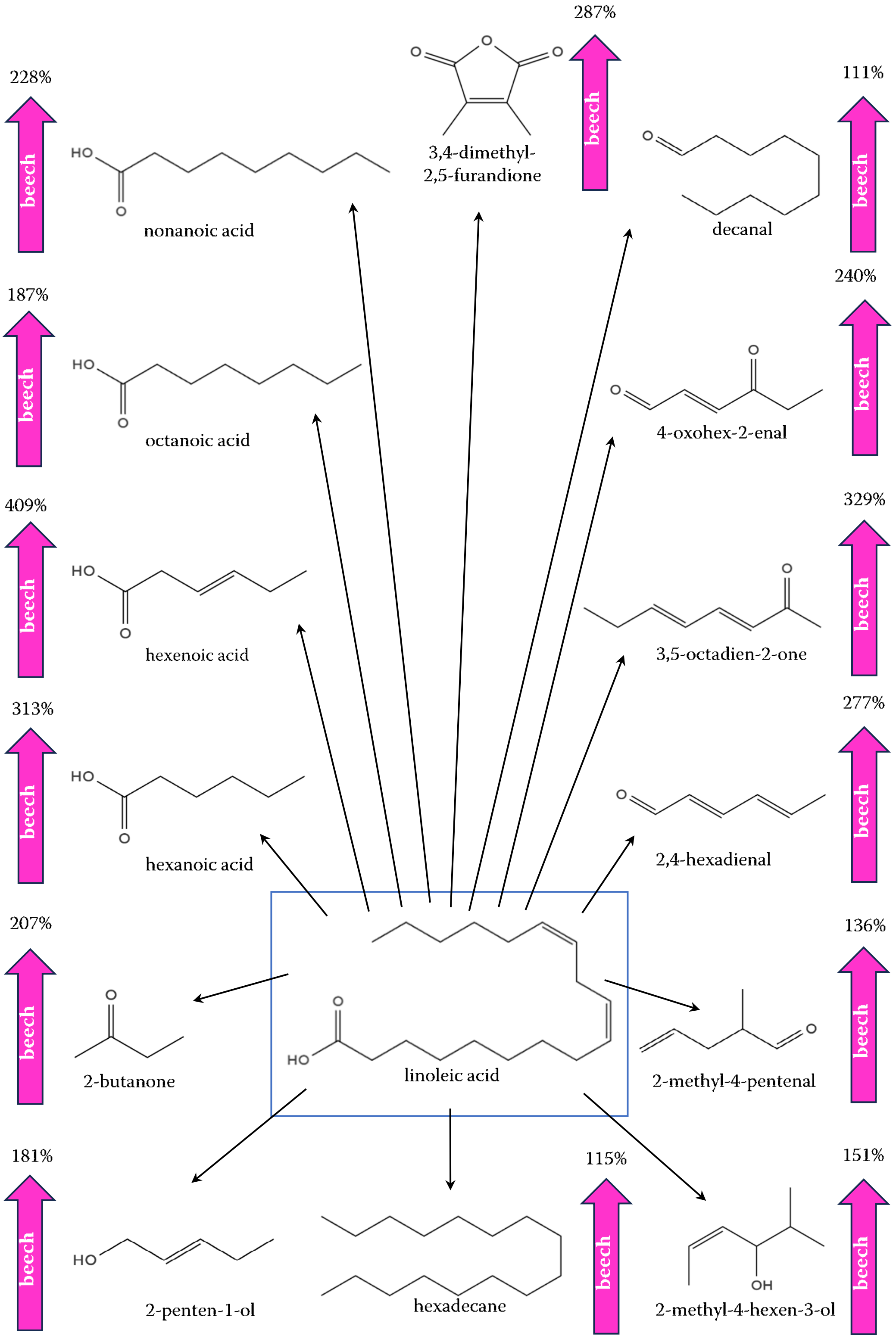

Figure 8. Increase or decrease of selected compounds associated with linoleic acid catabolism with significantly different production ($P < 0.1$) in leaves between uninfested and infested trees [beech (*Fagus sylvatica*)]

Arrow pointing upwards – an increase in compound production in infested trees; arrow pointing downwards – a decrease in compound production in infested trees; percentages above the arrow – the percentage increase or decrease in production of the compound in infested trees (100% = uninfested trees)

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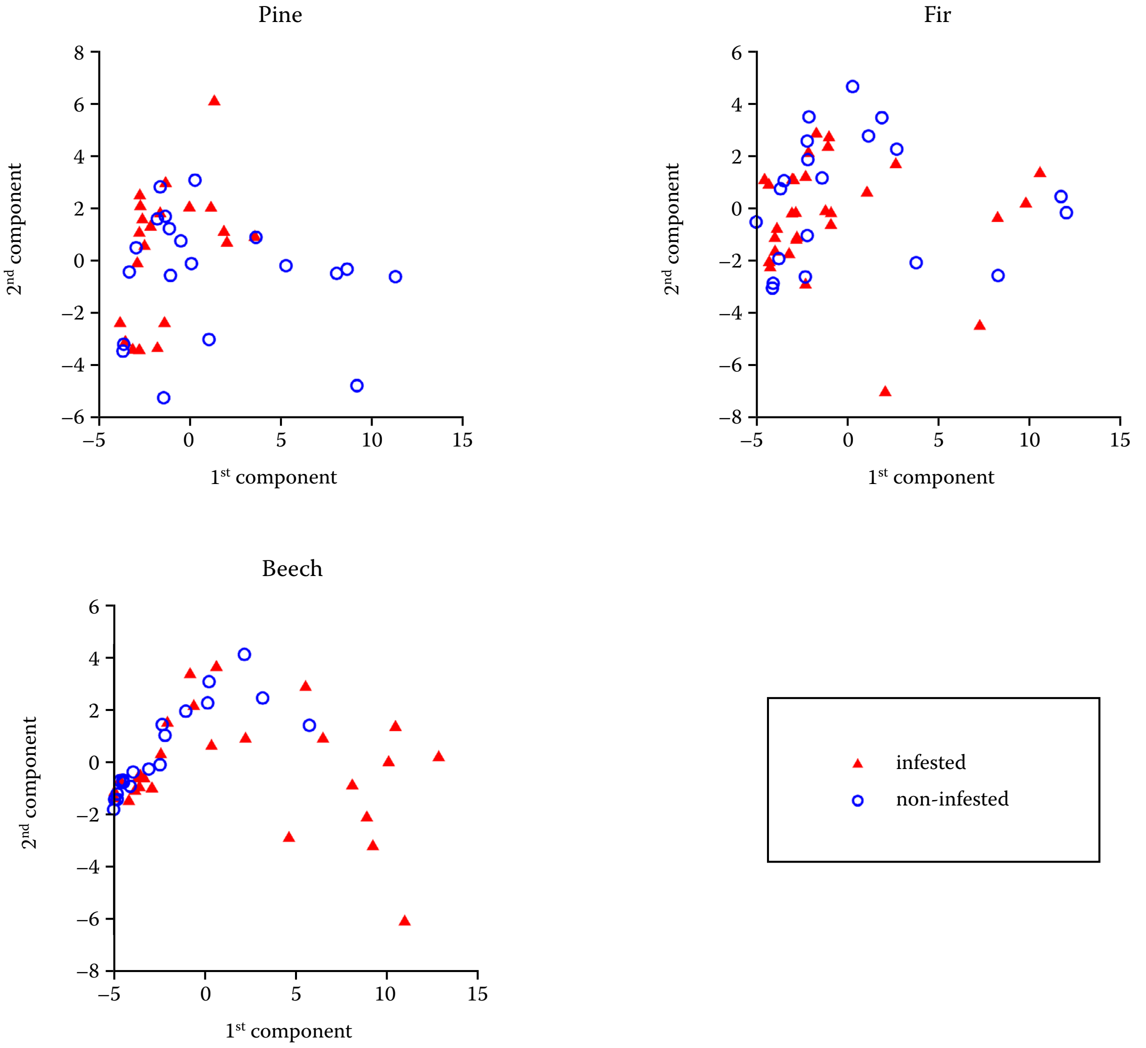

Figure 9. Results of PCA for each species

Each point in the graph represents one tree whose *x* and *y* coordinates are determined by the first two principal components obtained from locality-adjusted VOC data

neous. The beech data suggested homogeneity in uninfested trees. This homogeneity or heterogeneity may be partly explained by the chemotype. Recent studies have revealed considerable chemotypic diversity in the monoterpene profiles of various pine species. In Scots pine, three chemotypes characterised by high proportions of α-pinene, β-pinene, or limonene have been identified, with their distribution related to climatic variables (Taft et al. 2015). A study on four pine species (*P. halepensis*, *P. pinaster*, *P. pinea*, and *P. sylvestris*) showed the occurrence of chemotypes in three species, with *P. pinea* showing a homogeneous essential oil composition (Rodrigues et al. 2017). Similar chemotypic variations have been observed in wild cotton plants, with two distinct chemotypes showing geographic distribution patterns (Clancy et al. 2022). These chemotypic differences may influence plant-insect interactions and disease resistance. For example, in lodgepole pines, volatile communication and recognition of related species have been found to be chemotype-dependent, affecting tree responses to mountain pine beetle attack (Hussain et al. 2019). Understanding these chemotypic differences is crucial to predicting plant responses to biotic and abiotic stressors. Non-clustering may also be related to the presence of VOCs, which have high variability and are unrelated to infestation. As a result, the contribution of a few infestation-related compounds was not reflected in the PCA.

CONCLUSION

We investigated the changes in the terpene composition of surviving trees in bark beetle-infested stands of beech (*F. sylvatica*), pine (*P. sylvestris*), and fir (*A. alba*) in the Czech Republic. Our data showed that the distribution of different groups of volatile compounds varied according to the tree species. Conifers respond to beetle infestation by increasing the production of monoterpenes, decreasing the volatilisation of sesquiterpenes, and increasing the production of aliphatic alcohols, ketones, acids, esters, aldehydes, and phenolic compounds. In all three tree species, other substances with insignificant changes in production were also found. Understanding these complex chemical interactions is essential for the development of effective bark beetle management strategies, including the use of semiochemicals for pest control.

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