BRANCH-LOCALIZED INDUCTION PROMOTES EFFICACY OF VOLATILE DEFENCES AND HERBIVORE PREDATION IN TREES

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Abstract – Induction of plant defences can show various levels of localization, which can optimize their efficiency. Locally induced responses may be particularly important in large plants, such as trees, that show high variability in traits and herbivory rates across their canopies. We studied the branch-localized induction of polyphenols, volatiles (VOCs), and changes in leaf protein content in *Carpinus betulus* L., *Quercus robur* L., and *Tilia cordata* L. in a common garden experiment. To induce the trees, we treated ten individuals per species on one branch with methyl jasmonate. Five other individuals per species served as controls. We measured the traits in the treated branches, in control branches on treated trees, and in control trees. Additionally, we ran predation assays and caterpillar food-choice trials to assess the effects of the treatment on other trophic levels. Induced VOCs included mainly mono- and sesquiterpenes. Their production was strongly localized to the treated branches in all three tree species studied. Treated trees showed more predation events than control trees. The polyphenol levels and total protein content showed a limited response to the treatment. Yet, winter moth caterpillars preferred leaves from control branches over leaves from treated branches within *C. betulus* individuals and leaves from control *Q. robur* individuals over leaves from treated *Q. robur* individuals. Our results suggest that there is a significant level of localization in induction of VOCs and probably also in unknown traits with direct effects on herbivores. Such localization allows trees to upregulate defences wherever and whenever they are needed.

Key Words – Herbivory, hornbeam, indirect defences, linden, oak, methyl jasmonate, polyphenols, protein content, terpenes, VOCs

INTRODUCTION

Plants employ a bewildering diversity and variability of defence strategies. The efficiency of particular defences is highly dependent on the identity of herbivores and surrounding abiotic conditions (Defossez et al. 2018; Volf et al. 2018). Variability in defences and plasticity in their deployment thus help plants face a wide variety of challenges (Koricheva et al. 2004; Volf et al. 2019b). Induced defences are deployed in response to external stimuli, such as herbivory. They do not prevent the initial damage but can be upregulated when required, allowing plants to prioritize investment in defence over growth (Backmann et al. 2019). Such a strategy allows plants to respond to spatial and temporal changes in insect herbivore communities that require deploying specific defences whenever and wherever they are needed (Lämke and Unsicker 2018; Turlings and Erb 2018).

Induced defences involve several, partly complementary, mechanisms rendering them efficient against a broad spectrum of herbivores. After herbivore attack, plants can upregulate defences that are targeted directly at the herbivore. Direct defences involve increases in the levels of secondary metabolites, enzymes, trichome density or leaf toughness (Agrawal 1999; Barbehenn et al. 2009; Barton 2016). For example, induced increase of polyphenol oxidase (PPO) activity in mountain birch can promote resistance against *Epirrita autumnata* (Borkhausen) caterpillars (Ruuhola et al. 2008). Herbivory can also cause compensatory regrowth and shifts in leaf or stem nutrients, which makes the plant more or less attractive to herbivores (Utsumi and Ohgushi 2008). Such changes can be induced immediately or with a delay, which sometimes can be as long as the period until the following season (Rubert-Nason et al. 2015; Tuomi et al. 1988). In addition, plants can employ indirect defences, which attract predators and parasitoids. Indirect defences may involve the production of various volatile organic compounds (VOCs) (Clavijo McCormick et al. 2012; Dicke and Loon 2000; Turlings and Erb 2018). VOCs, such as terpenoids, or various green leaf volatiles including esters and alcohols, can be detectable by

predators and parasitoids even in complex environments (Vet et al. 1991). VOCs thus may play a prominent role in complex environments such as tree canopies, as they may help predators and parasitoids navigate efficiently through the dense foliage towards their prey (Amo et al. 2013; Turlings and Erb 2018).

Induced defence responses can show various levels of localization, which helps to further optimize their efficiency (Eyles et al. 2010). After herbivore or pathogen attack, the strongest and fastest induced responses are commonly observed at the site of attack (Mason et al. 2017; Piggott et al. 2004; Tuomi et al. 1988). Mostly, plant defences are also induced at the level of the whole plant. Such a systemic response elevates defence levels in hitherto undamaged plant parts (Eyles et al. 2010; Kachroo and Robin 2013). The strength of the response at more distal plant parts depends on how they are connected to the phloem or the distance from the attacked site if the induction relies on airborne signals (Heil and Ton 2008; Rubert-Nason et al. 2015; Viswanathan and Thaler 2004). Localized induced responses can be especially important in large plants, such as trees, as they can further help upregulate the defences where they are currently needed and can reduce resource investments in defence (Lämke and Unsicker 2018; Volf et al. 2020). Saving on the costs may be especially important in the case of energetically demanding chemical defences (Mason et al. 2017). Furthermore, as trees are large, they are more likely to experience localized herbivory (Mason et al. 2017), partly due to their complex architecture and spatial variation in their traits. Indeed, there is substantial variation in leaf traits, abiotic factors, and the number and types of biotic interactions among different sections of the canopy (Lämke and Unsicker 2018; Murakami et al. 2005; Rubert-Nason et al. 2015). For example, young and light-exposed leaves are more valuable for the plant because they are photosynthetically active. At the same time, such leaves are usually more attractive for herbivores due to their higher nutritive quality and lower lignin content (Schultz and Baldwin

1982). Allocating induction of defences primarily to parts of the canopy bearing young or photosynthetically active foliage can be beneficial for trees (Lämke and Unsicker 2018).

Induced responses in trees are highly variable among species, individuals and their parts (Lämke and Unsicker 2018; Neuvonen et al. 1987; Rubert-Nason et al. 2015). They range from being systemic to highly localized in individual leaves or needles (Bonello and Blodgett 2003; Eyles et al. 2010; Piggott et al. 2004; Rubert-Nason et al. 2015). Both systemic and highly localized induced responses may have direct and indirect effects on herbivores (Eyles et al. 2010; Lämke and Unsicker 2018). We hypothesize that localization of induced responses in individual branches (e.g. Tuomi et al. 1988) may be especially important in trees. First, it can help trees cope with the variation in abiotic and biotic factors within their canopy (Lämke and Unsicker 2018; Volf et al. 2020). Second, it balances the benefits of upregulating defences only when needed to upregulate defences in large enough modules. The latter is important to protect the attacked section against both sessile and mobile herbivores, while efficiently attracting their natural enemies to the damaged area.

In order to explore the extent and importance of branch-localized induction, we studied methyl jasmonate-induced responses in polyphenols, VOCs, and changes in nutritional value (leaf protein content) in a common garden experiment. We compared the responses among *Carpinus betulus* L. (European hornbeam), *Quercus robur* L. (common oak), and *Tilia cordata* Mill. (small-leaved lime) individuals to see if they are consistent among the three species. We supplemented the chemical analysis with predation assays, using clay caterpillars, and food-choice trials with generalist winter-moth caterpillars (*Operophtera brumata*). Combined, these experimental data allowed us to assess whether the induced responses had relevant ecological consequences. We expected i) branch-localized induction in all traits we measure, ii) increased predation rates on induced trees, and iii) lower preference of caterpillars for leaves from induced branches.

METHODS AND MATERIALS

The experiment was conducted between 10 May and 11 June 2018 in the Research Arboretum Großpösna (51°15'41"N, 12°29'55"E, ca 160 m a.s.l.) on an experimental plot that was set up by Ohse (2018). The plot includes 24 tree species with 32 replicates per species. The trees were planted in the winter of 2013/14 as two-years old saplings in a randomized grid design with 1.0 m gaps between individual plants. We selected 15 individuals of *Carpinus betulus* L. (average height (h) = 3.4 m, average diameter at breast height (DBH) = 1.6 cm), 15 individuals of *Quercus robur* L. (h = 3.0 m, DBH = 1.6 cm) and 15 individuals of *Tilia cordata* Mill. (h = 3.5 m, DBH = 1.9 cm) for our experiment. The closest distance between the selected neighbouring trees was 1.0 - 4.0 m. We avoided neighbouring conspecific trees and trees growing directly at the margins of the plot to avoid edge effects. All trees were treated with Promanal insecticide (Progema, Aerzen, Germany) on 15 March 2018 and with Spruzit insecticide (Neudorff, Emmerthal, Germany) on 25 April 2018 to minimize variation between the experimental trees due to naturally occurring herbivory prior to the experiment.

Induction experiment We randomly selected 5 individuals per tree species as controls and 10 individuals per species for the induction treatment. We selected three major branches in each tree (Fig. 1). The middle branches on the treated trees were used for an induction treatment (hereafter referred to as treated branches on treated trees - TT). The other two branches on the treated trees and all three branches on the control trees were used as two types of control (hereafter referred to as control branches on treated trees - CT and control branches on control trees - CC). This experimental set-up allowed us to distinguish between localized (upregulation in TT only) and systemic induction (upregulation in both TT and CT). The selected branches were 68-201 cm above ground level and were shaded by surrounding trees. In each branch, we selected its terminal part to be used in the experiments and for measurements of induced

177 responses. The terminal parts were ca 50 cm long and had similar numbers of leaves when
178 compared among conspecifics (*C. betulus* – 45 ± 17 leaves, *Q. robur* – 25 ± 14 leaves, *T.*
179 *cordata* – 31 ± 10 leaves, values are mean ± SD). We measured the shortest distance between
180 the studied branches (as the distance between their closest leaves on a straight line) and their
181 distance over the trunk (as their distance over trunk and basal parts of the respective branches).

182 We treated TT branches with methyl jasmonate (MeJA). MeJA is commonly used to simulate
183 herbivory damage by chewing insects in ecological experiments (e.g. Mrazova and Sam 2018).
184 MeJA typically induces a broad and largely unspecific spectrum of responses. While it does not
185 provide the same level of specificity and intensity of induction as a treatment with real
186 herbivory, it allows for relative comparisons between plants or their parts. It also allows to
187 induce plant responses in a standardized manner across a large number of branches and can
188 reduce potential variation in induced responses due to naturally occurring herbivory (Klimm et
189 al. 2020). Here, we use it as a proxy for comparing general inducibility and the level of
190 localization in induction in the three tree species and their branches.

191 We enclosed TT branches in clear 45 x 55 cm polyamide bags (Studio Cook BV, Zeewolde,
192 NL) and put 9 µl of MeJA (Purity 95% Sigma-Aldrich, St. Louis, Missouri, US) on a cotton
193 ball (Hartmann, Heidenheim, DE) inside. CT and CC branches received bags with cotton
194 without MeJA. We left the branches enclosed in the bags for 24 hours. We then removed the
195 bags and measured volatiles or placed clay caterpillars for predation experiments (Fig. 1). The
196 induction treatment was repeated 11 times, typically at three-day intervals to allow enough time
197 for the predation experiment and to explore changes over time. Only after the first VOC
198 sampling, we repeated the induction at a shorter interval before we placed clay caterpillars on
199 the branches (Fig. 1).

200 The experiment started on 10 May and was carried out until 11 June 2018. By that time, we
201 observed that some of the trees tend to suffer from severe water stress because of the drought

that affected the region in 2018. Therefore, we selected 8 tree individuals per species without obvious drought stress symptoms (5 treated and 3 control individuals), re-measured their volatile production, and stopped all experimental work with all the studied trees in the field to avoid the effects of drought on our results.

VOC sampling and analysis We passively sampled VOCs using polydimethylsiloxane (PDMS) tubes (Carl Roth GmbH, Karlsruhe, Germany) from all studied branches following Kallenbach et al. (2014) to compare their production. We placed two clean 1.5 cm PDMS cuttings (technical replicates) on a stainless-steel wire, attached them to the measured branch and enclosed the branch and tubes in a 45 x 55 cm polyamide bag. The VOCs were passively adsorbed to the PDMS cuttings from the headspace for 24 hours. We carried out the sampling twice: after the first induction treatment (initial sampling; all trees sampled) and approx. one month later after 11 induction treatments (final sampling; eight trees per species sampled) once the experiment came to a halt because of the drought. In both cases, we waited ca 30 minutes after removing the bags used for the induction treatment before we put new bags on the branches to sample VOCs.

We performed gas chromatography to quantify the sampled volatiles. The PDMS cuttings were analysed by a thermal desorption-gas chromatograph-mass spectrometer (TD-GC-MS). The TD-GC-MS consisted of a thermodesorption unit (MARKES, Unity 2, Llantrisant, UK) and an autosampler (MARKES, Ultra 50/50). PDMS cuttings were transferred to empty stainless steel tubes (MARKES), and then desorbed with helium as carrier gas and a flow path temperature of 160 °C using the following conditions: Dry Purge 5 min at 20 ml/min and Pre Purge 2 min at 10 ml/min to remove remaining water, Desorption 8 min at 200 °C with 60 ml/min, Pre Trap fire purge 1 min at 60 ml/min, Trap heated from 0 to 300 °C at maximum speed and hold for 4 min. The volatiles were separated on a gas chromatograph (Bruker, GC-456, Bremen,

Germany) connected to a triple-quad mass spectrometer (Bruker, SCION) equipped with a DB-WAX column: (30m x 0.25mm inner diameter x 0.25um film thickness, Restek). The temperature program was set to the following: 60 °C (hold 2 min), 30 °C/min to 150 °C, 10 °C/min to 200 °C and 30 °C/min to 230 °C (hold 5 min). Helium was used as carrier gas at a constant flow rate of 1 ml/mi. MS conditions were set to 40 °C for the manifold, 240 °C at the transfer line and 220 °C for the ion source. The scan-range was 33 –500 m/z for a full scan and scan-time was 250 ms. We selected the most prominent peaks in the chromatograms and set signal to noise ratio to > 10. Peaks that were also present in the chromatograms of empty stainless-steel tubes were regarded as systemic contamination and were excluded from further analysis. VOCs that responded to our treatment were tentatively identified by comparison to the NIST database and comparison to retention indices from the literature. The peak areas of these compounds were calculated using the Bruker Workstation software (v8.0.1).

Measurements of polyphenols and protein content We sampled leaves from the studied branches to quantify induced changes in polyphenols, protein content, and leaf palatability to caterpillars (see *Caterpillar choice assays* below). Using a scalpel to minimize the wound, we sampled the first two youngest fully developed leaves from all TT branches, upper CT branches, and middle CC branches (a single leaf per branch was sampled in the case of *Q. robur*, which had sufficiently large leaves). The leaves were sampled after four (*T. cordata*) or five (*C. betulus* and *Q. robur*) induction treatments (10 and 14 days after the first treatment, respectively) in order to time their sampling with the caterpillar hatching in our colony.

Half of the sampled leaf material (avoiding the central vein) was freeze-dried and homogenized to analyse polyphenol profiles and protein content. Polyphenols were extracted from ca 20 mg (in 0.01 mg accuracy) of homogenized material using 80:20 (v/v) acetone/water solvent as described in detail in Malisch et al. (2016). We ran two separate sets of assays to analyse

252 polyphenols. Firstly, we quantified total content for each of the main polyphenol sub-groups
253 (in mg/g) by UPLC-QqQ-MS/MS with the methods of Engström *et al.* (2014; 2015) as
254 described in e.g. Malisch et al. (2016). With these methods polyphenols are first separated by
255 UPLC and then each polyphenol is fragmented in the MS ion source to produce compound
256 group-specific fragments that are detected by the group-specific MS/MS methods created for
257 each polyphenol group separately (see Salminen (2018) for further details). This technology
258 enables even the determination of the sub-unit composition (procyanidin and prodelphinidin
259 units) and molecular size (mean degree of polymerization) of polymeric proanthocyanidins.
260 The measured polyphenol sub-groups included (1) galloyl and hexahydroxydiphenoyl
261 (ellagitannins, HHDP) units found in hydrolysable tannins, (2) procyanidin and prodelphinidin
262 units found in proanthocyanidins, (3) kaempferol, quercetin and myricetin units found in
263 flavonol glycosides, and (4) quinic acid units found in quinic acid derivatives such as caffeoyl
264 and coumaroyl quinic acids. Compound groups were quantified by using pentagalloylglucose
265 (galloyl units), tellimagrandin I (HHDP units), kaempferol-3-O-glucoside (kaempferol units),
266 quercetin-3-O-glucoside (quercetin units), myricetin-3-O-rhamnoside (myricetin units),
267 purified procyanidin-rich proanthocyanidin fraction (procyanidin units), purified
268 prodelphinidin-rich proanthocyanidin fraction (prodelphinidin units), and chlorogenic acid
269 (quinic acid units) as external standards. Secondly, we ran two activity assays to quantify the
270 two major functions of polyphenols in anti-herbivore protection – oxidative activity and protein
271 precipitation capacity. Polyphenol oxidative activity was measured following Salminen &
272 Karonen (2011) using gallic acid as the standard. Protein precipitation capacity was measured
273 following Hagerman's radial diffusion assay (Hagerman and Butler 1978) using
274 pentagalloylglucose as the standard. Both assays gave activities in the unit of mg/g.

275 The protein content was measured with Bradford colorimetric assay (Kruger 2009). We
276 extracted the proteins from 3-6 mg of the dried leaf material with TCA/acetone solution

277 following Jorge et al. (2005). We dissolved the protein in 200µl 8M Urea/2% Chaps (Carl Roth
278 GmbH, Karlsruhe, Germany) and added 34 µl of this solution to 1 ml of Bradford reagent
279 solution and measured the protein content using V-630 UV-Vis spectrophotometer (Jasco,
280 Easton, MD, USA).

281

282 *Predation levels* We used clay caterpillars to estimate changes in predation rates in response to
283 the MeJA treatment. This method is widely used to estimate predation rates by both vertebrate
284 and invertebrate predators. Although it has some limitations (i.e. plasticine caterpillars are
285 immobile, they do not produce the same cues as real lepidopteran larvae etc.) and may inflate
286 real absolute predation rates, this method provides conservative estimates of relative predation
287 pressure comparable across treatments (Howe et al. 2009). It allows placing large number of
288 replicates and leaving them in the field for several days, which is particularly important in
289 temperate habitats where the predation rates are generally low. We prepared the caterpillars (3
290 mm in diameter, 3 cm in length) using non-toxic Newplast plasticine (Newclay Products,
291 Newton Abbot, UK). We attached 5 caterpillars to twigs with Loctite 401 super glue (Henkel,
292 Düsseldorf, DE) for each studied tree (1 to each experimental branch and 2 to additional
293 branches; 225 clay caterpillars placed in total) following each induction treatment. The
294 caterpillars were first placed on 13 May. They were exposed to predators for 48 hours, checked
295 for predation marks before the next induction treatment following Howe et al. (2009). Damaged
296 caterpillars were either replaced or fixed. The last checking was on 9 June. We classified the
297 predation marks as bird or invertebrate predation. Marks caused by surrounding twigs and
298 leaves or by invertebrates which do not prey on caterpillars (i.e. snails) were excluded.

299 We noticed that predation rates inside our experimental plot, where the canopy density reduced
300 light availability, were low. Therefore, we placed 75 additional clay caterpillars on the branches
301 at the margin of the plot for a comparison. The caterpillars were placed in ca 2 m intervals, 1.5

m above ground level on randomly selected trees of various species. The clay caterpillars were checked in the same intervals and on the same days as the caterpillars inside the plot. As the decision to place these caterpillars was made directly before the experiment started, we did not have a chance to treat these trees with insecticides. We thus avoided placing the clay caterpillars on trees showing high herbivory damage to lower possible effects of naturally occurring herbivory. We compared the light intensity between the trees inside the plot and on the margin at 1.2 m height above ground using Quantum Sensor LI-190 (LI-COR, Lincoln, Nebraska, USA) on 6 June 2018 between 9:30 and 10:15 when the sky was clear.

We temporally ceased both the induction treatment and clay caterpillar exposure for a period of three days (22– 24 May 2018) to conduct the food-choice trials with *O. brumata* caterpillars.

Caterpillar choice assays We used the second half of the sampled leaf material for food-choice trials to explore the effects of induction treatment on the leaf palatability to winter moth caterpillars (*Operophtera brumata* L.). *O. brumata* is a polyphagous species feeding on all three species studied and it commonly occurs in the region. *O. brumata* females were obtained in November 2017 near České Budějovice, CZ. After oviposition, the eggs were stored at 2 °C to overwinter. The eggs were transferred to 14 °C 14:10 light conditions at the end of April. The larvae fed on *T. cordata* leaves until their last instar. Since their previous experience with non-treated *T. cordata* leaves could affect their preference, we removed *T. cordata* from the food-choice trials and used *C. betulus* and *Q. robur* only. To run the two-choice trials with *O. brumata* caterpillars, we prepared leaf discs of 1.5 cm in diameter from the sampled leaves, while avoiding the mid vein. In a 'branch comparison', each larva was offered a disc from a TT branch and a disc from a CT branch from the same tree. We repeated food-choice trials for each treated *Carpinus* and *Quercus* individual twice (40 last-instar larvae used in total). In a 'tree comparison', each larva was offered a disc from a TT branch and a CC branch on a control tree.

Each control tree was compared to four randomly selected treated trees (40 last-instar larvae used in total). All larvae were allowed to feed for 7 hours. Then the leaf discs were photographed and the remaining leaf area was measured in imageJ (Abràmoff et al. 2004).

Statistical analysis First, we analysed the effect of the induction treatment on the production of VOCs during the initial and final sampling for each tree species separately. We used Principal Component Analysis (PCA) to explore the variation in the overall VOC profiles among the studied branches. We then tested the effect of our treatment on the VOC profiles with *Redundancy Analysis* (RDA). We used the treatment (TT, CT, and CC) as explanatory variable and tree individual as a covariable defining the permutation blocks. The significance of all canonical axes was tested using 9999 permutations and the explained variability was adjusted following ter Braak and Smilauer (2012). The multivariate analyses were performed in CANOCO 5 (ter Braak and Smilauer 2012). Furthermore, we used *Linear Mixed Effect Models* (LMEs) in R 3.6.1 (R Core Team 2019) using 'lme4' package (Bates et al. 2015) to confirm the trends in individual volatiles. We used the treatment as a fixed effect and tree individual as a random factor. Due to the high number of individual compounds involved in the comparisons, we applied Bonferroni correction to the results of LME models comparing production of individual volatiles across the treatments. We used log-transformed peak areas representing individual compounds in all analyses. In case we found a significant treatment effect, we performed a post-hoc test using the function 'difflsmeans' in 'lmerTest' R package (Kuznetsova et al. 2017), specifically looking for locally or systemically induced compounds. We used the following definitions of locally and systemically induced compounds based on their production by the studied branches:

Locally induced compounds: TT > CT & TT > CC & CT = CC

Systemically induced compounds: TT > CC & CT > CC

Furthermore, we used LMEs to test if the total VOC production in CT branches was correlated with their distance from TT branches. We used the shortest distance between the branches and their distance over trunk as a fixed effects and tree identity as a random factor in our LMEs.

Second, we analysed if the total polyphenol content, polyphenol diversity (measured as Shannon-index based on the content of individual sub-groups), production of individual polyphenol sub-groups, polyphenol protein precipitation capacity, polyphenol oxidative activity, and total protein content differed between CC, CT, and TT branches using LMEs. We performed the analysis for each tree species separately and used tree individual as a random factor. In case we found a significant effect of the treatment, we performed a post-hoc test using the function 'difflsmeans' (Kuznetsova et al. 2017). We used log-transformation to normalize polyphenol content and activity data (in mg/g). We used arcsine transformation to normalize the relative share of procyanid and prodelphinidin units of proanthocyanidins (in %).

Third, we tested if the treatment had any effect on predation rates. We compared the total number of predated clay caterpillars between the treated and control trees within our plot and between CT and TT branches using linear models and LMEs, respectively. When comparing the treated and control trees, we summarized the total number of predation events from all branches in individual trees and log-transformed it. We then created a null model including the tree species (to account for its effect) and compared it with a model including both the tree species and induction treatment as explanatory variables based on their AIC. When comparing CT and TT branches with LMEs, we compared a null model including the tree species as a fixed effect with a model including both the tree species and treatment as fixed effects based on their AIC. Both the null and tested model included the tree individual as a random factor. Furthermore, we calculated the average predation rates over 48 hours for caterpillars placed inside our plot and on its margin to see if there was any difference in predation rates.

Fourth, we analysed the effects of the treatment on caterpillar preference in the food-choice trials. For each leaf disc, we quantified the proportion of its area eaten and normalized it with arcsine transformation. The proportion of disc area eaten was compared among the treatments using LMEs. In 'branch comparison', we used branch treatment (TT vs. CT) as a fixed effect, and the tree individual and trial (to link the discs from same trials) as nested random factors. In 'tree comparison', we used tree treatment (TT vs. CC) as a fixed effect and the control tree individual involved in the particular comparison and trial as nested random factors. We analysed the results for *C. betulus* and *Q. robur* separately.

RESULTS

All together, we analysed 27 volatiles in *Carpinus betulus*, 59 volatiles in *Quercus robur*, and 46 volatiles in *Tilia cordata* (Table S1). Most of the classified volatiles belonged to the mono- and sesquiterpenes, although other classes were also recorded.

In our initial sampling directly after the first MeJA treatment, PCA analysis only revealed a limited separation between CC (control branches on control trees), CT (control branches on treated trees), and TT (treated branches on treated trees) in terms of their volatile profiles (Fig. 2). RDA showed that MeJA treatment had a significant effect in the case of *C. betulus* (pseudo-F = 7.6, p = 0.001, 18.1% of adjusted variability explained) and *Q. robur* (pseudo-F = 3.1, p = 0.001, 7.1% of adjusted variability explained). Its effect on *T. cordata* was not significant (pseudo-F = 1.8, p = 0.182, 2.7% of adjusted variability explained). Four individual compounds, two mono- and two sesquiterpenes, responded to the MeJA treatment in *C. betulus* when analysed with LMEs and applying Bonferroni correction. All four showed a localized induction. One monoterpene, trans-ß- ocimene, increased significantly upon MeJA treatment in *Q. robur*, showing a localized induction. There was no compound showing a significant response to MeJA treatment in *T. cordata* (Table S2).

For the VOCs collected at our final sampling after 11 MeJA treatments, PCA analysis revealed a strong separation in volatile profiles between CC and CT branches on the one side and TT branches on the other (Fig. 2). RDA showed that the treatment had a significant effect in the case of *C. betulus* (pseudo-F = 10.8, p = 0.007, 38.0% of adjusted variability explained), *Q. robur* (pseudo-F = 32.0, p = 0.005, 66.0% of adjusted variability explained), and *T. cordata* (pseudo-F = 17.6, p = 0.013, 52.6% of adjusted variability explained). Ten individual compounds responded to MeJA treatment in *C. betulus* when analysed with LMEs and applying Bonferroni correction. In addition to the four terpenoids induced after one treatment, the emission of six more sesquiterpenes significantly increased in MeJA treated branches. Depending on the compound, the emission on TT branches was 17-1000 times more compared to that of CC branches (Fig. 3). In *Q. robur*, 45 compounds responded to the MeJA treatment. The majority of compounds that could be identified were terpenes, four mono- and 21 sesquiterpenes or derivatives thereof. But we also found an aldehyde (Q3), an alkatetraene (Q10), two alcohols (Q18, Q47) and an ester (Q41) that were strongly induced by MeJA. Out of these, 36 showed localized induction, one showed systemic induction, and eight showed other trends. In *T. cordata*, 27 compounds responded to the treatment (Table S2). The ones we identified included eight mono- and 12 sesquiterpenes or derivatives thereof, and one benzoate (T45). Out of the compounds showing a significant response to the treatment in *T. cordata*, 21 showed localized induction, one showed systemic induction, and five showed other trends. Considering all three tree species, sesquiterpenes were most frequently induced in TT branches when compared to CC branches (Fig. 3). Whereas TT branches of *C. betulus* and *T. cordata* produced only one new compound after 11 MeJA treatments, we found 15 compounds in the VOC profiles of *Q. robur* TT branches that were produced *de novo* (Fig. 3).

The volatile production in CT branches was generally not correlated to their distance to TT branches. The only two exceptions were i) a negative correlation between the total VOC

426 production in CT branches and their shortest distance to TT branches in the case of our initial
427 sampling from *Q. robur* ($\chi^2(1) = 4.07$, $p = 0.044$) and ii) a positive correlation between the total
428 volatile production in CT branches and their trunk distance to TT in the case of our final
429 sampling from *C. betulus* ($\chi^2(1) = 5.97$, $p = 0.015$).

430 The polyphenol profiles, polyphenol activities, or total protein content generally did not show
431 a significant response to the MeJA treatment (Table S3). The only exception was the percentage
432 of procyanidin units in the total proanthocyanidin content that was significantly different
433 between the control branches on treated trees and treated branches on treated trees in *Q. robur*
434 ($\chi^2(2) = 6.25$, $p = 0.044$). In *T. cordata*, the content of kaempferols showed a marginally
435 significant response to the treatment ($\chi^2(2) = 5.33$, $p= 0.0696$). In *C. betulus*, neither
436 polyphenols nor protein content showed a significant response to the treatment.

437 We recorded 98 predation events inside the plot over the entire period (Fig. S1). Only eight
438 predation events could be attributed to birds. Other clay caterpillars were either predated on by
439 invertebrates or it was not possible to distinguish whether the mark was caused by a bird or by
440 an invertebrate. The average number of predation events was higher on the treated trees than
441 on the control trees ($F_1 = 5.04$, $p = 0.030$; Fig. 4). There was no difference between predation
442 on CT and TT branches ($\chi^2(1) = 0.94$, $p = 0.333$). Due to low predation rates inside the plot, we
443 also set up an additional experiment to assess predation rates at the margins over 48 hours.
444 Inside the plot, 5.45% (0.44% by birds) of the clay caterpillars showed predation marks. At the
445 plot margin, the overall predation rate was 13.77% (3.39% by birds). The light intensity
446 measured at individual branches inside the plot (18.7±29.8 µmol s-1 m-2 per µA) was ca 2.3%
447 of the levels on its margin (798.7±555.9 µmol s-1 m-2 per µA).

448 The MeJA treatment affected caterpillar preference in the food-choice trials (Fig. 5). In the case
449 of *C. betulus*, the caterpillars preferred the discs from CC branches in 'tree comparison' ($\chi^2(1)$
450 $= 4.22$, $p = 0.040$) and the discs from CT branches in 'branch comparison' ($\chi^2(1) = 6.36$,

p=0.012) over the discs from TT branches. In the case of *Q. robur*, the caterpillars preferred the discs from CC branches over the discs from TT branches in 'tree comparison' ($\chi^2(1) = 5.93$, p=0.015). However, they did not differentiate between the leaf discs from CT and TT branches in the 'branch comparison'($\chi^2(1) = 1.14$, $p = 0.286$). In fact, they consumed very little of either disc category (Fig. 5).

DISCUSSION

We analysed induction of several responses with possible direct and indirect effects on herbivores and their localization to individual branches in three different tree species. Our results show that various tree traits differ in their inducibility and level of localization (Clavijo McCormick et al. 2014; Mason et al. 2017). When induced with MeJA, which we used as a proxy for induction by chewing herbivore damage, the induction of VOCs was strongly localized to individual branches. In contrast, we detected much weaker or no response in leaf polyphenols and protein content.

VOC production varies substantially among tree species, tree individuals or even their parts (Clavijo McCormick et al. 2014; Lämke and Unsicker 2018). Generally, inducibility should be higher in early successional or fast growing plants (Rasmann et al. 2011). Here, we recorded the highest diversity and inducibility of VOCs in *Q. robur*, a light demanding and long-lived pioneer species. High VOC diversity and intraspecific variation has been shown to reduce herbivory damage in patches of shrubs such as *Piper* (Salazar et al. 2016), possibly by confounding cues to specialized herbivores or providing new ones to predators. High diversity and variation of VOCs across canopies of large and long-lived trees, such as oaks, that harbour diverse insect assemblages could theoretically play a similar role (Lämke and Unsicker 2018; Volf et al. 2020). We recorded a systemic upregulation in two VOCs, suggesting a signal transfer by phloem or by airborne signals (Heil and Ton 2008; Viswanathan and Thaler 2004).

476 However, most of the compounds were upregulated in treated branches only. With one
477 exception, we also did not detect a negative correlation between the overall VOC production in
478 control branches and their distance to the treated branches, i.e. the control branches close to the
479 treated ones did not generally produce more VOCs.

480 Terpenoids, in particular sequiterpenes, were the most frequently locally induced compounds,
481 some also detected *de novo* in treated branches. The largely localized upregulation of terpenoid
482 VOCs is similar to the results of Clavijo McCormick et al. (2014) who recovered similar trends
483 in young poplars induced with herbivory. A highly localized induction of monoterpenes and
484 sesquiterpenes has been recorded also in red pine phloem. This observation, together with the
485 results presented here, suggest an important role of short-distance signalling for eliciting
486 biosynthesis of terpenoids in trees (Mason et al. 2017). In general, such short-term signaling
487 and localized induction responses allow individual tree modules to respond to steep
488 environmental gradients within canopies, maximizing their functioning at the whole-canopy
489 level.

490 Terpenoids are commonly induced by herbivore feeding in a wide range of plant species,
491 including trees. The quantity and type of terpenoids that are induced, may depend on the
492 herbivore species that are feeding or the type of damage they cause (Danner et al. 2018;
493 Unsicker et al. 2015). As such, herbivore-induced terpenoids are important infochemicals to a
494 wide range of predators and parasitoids that use them as cues to identify their specific prey or
495 host. In particular, *de novo* synthesized terpenoids are reliable cues as they are produced only
496 when the leaves are actually damaged (Vet et al. 1991). For example, blends of sesquiterpenes
497 can attract birds even in the absence of visual cues or when emitted by individual branches
498 (Amo et al. 2013; Mäntylä et al. 2017). Our results suggest that upregulation of VOCs localized
499 into a single branch could contribute to predator attraction in the system we studied. In large
500 trees, such a localized attraction can facilitate a faster herbivore removal by predators,

especially in case of patchy and aggregated herbivore distribution across the canopy (Travis and Palmer 2005). However, studying an increase in predation rates following an induction event in large mature trees would be required to reveal the level of localization in predation across tree canopies. Indeed, here the predators extended their search for prey to the whole canopies of our relatively small trees where the closest distance between branches was ca 45 cm, resulting into no difference in predation rates between CT and TT branches.

It was proposed that VOCs can serve an important role in complex environments with limited visibility and help natural enemies to navigate towards herbivores (Vet et al. 1991). We observed increased predation on treated trees in our densely vegetated plot where the light intensity was less than 3% of the levels of light intensity on its margin. This increase in predation rates provides some support for the hypothesis. However, the overall predation rates inside the plot were less than half that on its margin, although no trees on the margin were induced. The trend was even more pronounced when bird predation was considered separately as we observed ca 8x more predation events by birds on the plot margin. The difference in predation rates between the plot and its margin illustrates that abiotic factors such as light intensity, temperature, or habitat openness can strongly modify predation rates (Posa et al. 2007; Seifert et al. 2016). Additionally, the canopy structure and abiotic conditions can modify how VOCs spread, affecting their efficiency (Douma et al. 2019). While VOCs thus can serve their role as indirect defences, even in a dense jungle of foliage, their ecological relevance depends on factors affecting predators and the physical structure and accessibility of the habitat. The efficiency may also differ between VOC types (Douma et al. 2019). Here, we recorded primarily terpenoid based VOCs. Other types of VOCs such as green leaf volatiles, including esters and alcohols that were only marginally represented in our samples, can also play an important role in other systems (Clavijo McCormick et al. 2012).

525 In contrast to the strong induction of VOCs, our induction treatment elicited only a limited
526 change in proanthocyanidins and no changes in other polyphenols or in the protein content.
527 Proanthocyanidins show low protein precipitation and oxidative activities in the caterpillar mid-
528 gut (Salminen and Karonen 2011). Therefore, the shift in proanthocyanidin composition we
529 recorded in *Q. robur* has relatively low potential to serve as a form of induced resistance against
530 caterpillars or similar herbivores. Still, winter moth caterpillars preferred leaf discs of untreated
531 leaves of *Q. robur* and also of *C. betulus* over those punched from induced leaves, suggesting
532 either systemic (*Q. robur*) or branch-localized (*C. betulus*) differences in leaf quality.
533 Therefore, there was probably a trait, or a combination of traits, other than polyphenols
534 primarily responsible for the patterns in caterpillar food-choice in our study. We recorded a
535 significant upregulation of various mono- and sesquiterpene VOCs in the treated branches.
536 Possibly, the production of other bioactive terpenoids with higher molecular mass that are
537 contained in the leaves of studied species (Frédérich et al. 2009) could be upregulated as well,
538 explaining the trends in caterpillar preference we observed. Additionally, induced responses in
539 plants may involve a number of changes, including changes in physical traits, such as changes
540 in trichome density, that may have further contributed to the trends observed (Barton 2016).

541 The absence of a strong response in polyphenols to our induction treatment can result from the
542 relatively short period between the treatment and the time they were measured. The induction
543 of responses with direct effects on herbivores in herbaceous plants can be relatively rapid,
544 leading to an upregulation of defensive metabolites over a course of several days (van Dam et
545 al. 2004). While a relatively rapid upregulation of polyphenol-based defences has been recorded
546 in trees (Rubert-Nason et al. 2015; Ruuhola et al. 2008), other studies on trees also reported an
547 upregulation of polyphenols spanning over several months or seasons (Tuomi et al. 1988). Such
548 a delayed induced resistance can impose negative effects on older instars of herbivores with
549 long developmental times, herbivores occurring later in the season or possibly next generation

of the same species in the following year (Eyles et al. 2010; Roden and Mattson 2008; Tuomi et al. 1988). However, in temperate regions, herbivore abundance on trees shows strong seasonality, with many herbivore species undergoing a rapid development in spring. The spring peak in caterpillar abundance can last only two weeks (Volf et al. 2019a). Combining the relatively slow induction of direct defences with a fast induction of VOCs attracting natural enemies of herbivores can thus be an important defensive strategy. Higher light intensity and canopy openness during leaf flushing can contribute towards the importance of VOCs during that period. Furthermore, releasing VOCs by damaged foliage can prime the surrounding branches and trees for a faster response to herbivory occurring later in the season (Kim and Felton 2013). Thus, there may be a seasonal shift in the relative importance of traits with direct and indirect effects on herbivores. More readily inducible VOCs may be more important early in the season when canopies are open and young leaves produce VOCs in higher quantitates (Rostás and Eggert 2008). On the other hand, some of the polyphenol sub-groups, such as proanthocyanidins, may become more important in the later season once they accumulate (Salminen et al. 2004).

In conclusion, our results show that induction of VOCs can be localized to individual branches even in relatively small trees, thereby contributing to increased predation rates and herbivore removal. Additionally, our experiments suggest that localized changes in branches can also affect caterpillar preference in some tree species. In addition to helping trees to cope with steep environmental gradients across their canopies, such localized changes may have some potential to promote spatial chemical variation across the canopy (Lämke and Unsicker 2018; Rubert-Nason et al. 2015). Effects of this chemical variation can cascade to higher trophic levels, possibly promoting spatial variation in the communities of herbivores, predators, or parasitoids (Volf et al. 2020). Extending similar projects on localized induction to large canopy trees and

574 studying them within and across seasons could bring insights not only into plant defensive
575 strategies, but also into factors structuring diverse assemblages of multiple canopy organisms.

576

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588

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765 FIGURE CAPTIONS

766 Figure 1. Experimental design and timeline. We selected 15 trees per species. Ten were
767 subjected to our treatment, five were used as controls. In each tree, we selected three major
768 branches. The middle branches on the treated trees were treated with MeJA (TT branches). The
769 other two branches on the treated trees and all three branches on the control trees were used as
770 two types of control (CT and CC branches). The example of our timeline gives an overview of
771 the first nine days of our experiment. The experiment started with an induction treatment
772 followed by the first VOC sampling. After that, we repeated the induction treatment before
773 placing clay caterpillars to measure predation rates. The caterpillars were placed for 48 hours.
774 Then they were checked for predation marks, the induction was repeated, and the caterpillars
775 were placed again.

776 Figure 2. Variation in volatile organic compound (VOC) profiles between the studied branches
777 as visualized by PCA. In the case of initial sampling, PCA analysis revealed only a limited
778 separation in VOC profiles between treated and control branches in *Carpinus betulus* (A, first
779 two unconstrained axes explained 63.3 % of the variation in VOCs), *Quercus robur* (B, first
780 two unconstrained axes explained 47.4 % of variation), and *Tilia cordata* (C, first two
781 unconstrained axes explained 61.2 % of the variation in VOCs), although the effect of treatment
782 was significant in *C. betulus* and *Q. robur* when subsequently analysed by RDA. In the case of
783 the final sampling, treated branches on treated trees showed clearly different VOC profiles,
784 suggesting a strongly localized induction in *C. betulus* (D, first two unconstrained axes
785 explained 78.6 % of the variation in VOCs), *Quercus* (E, first two unconstrained axes explained
786 70.4 % of the variation in VOCs), and *Tilia* (F, first two unconstrained axes explained 77.3 %
787 of the variation in VOCs) that was also confirmed by the subsequent RDA. Branches are shown
788 as circles and the treatment is colour coded. VOCs are shown as arrows. Black arrows indicate

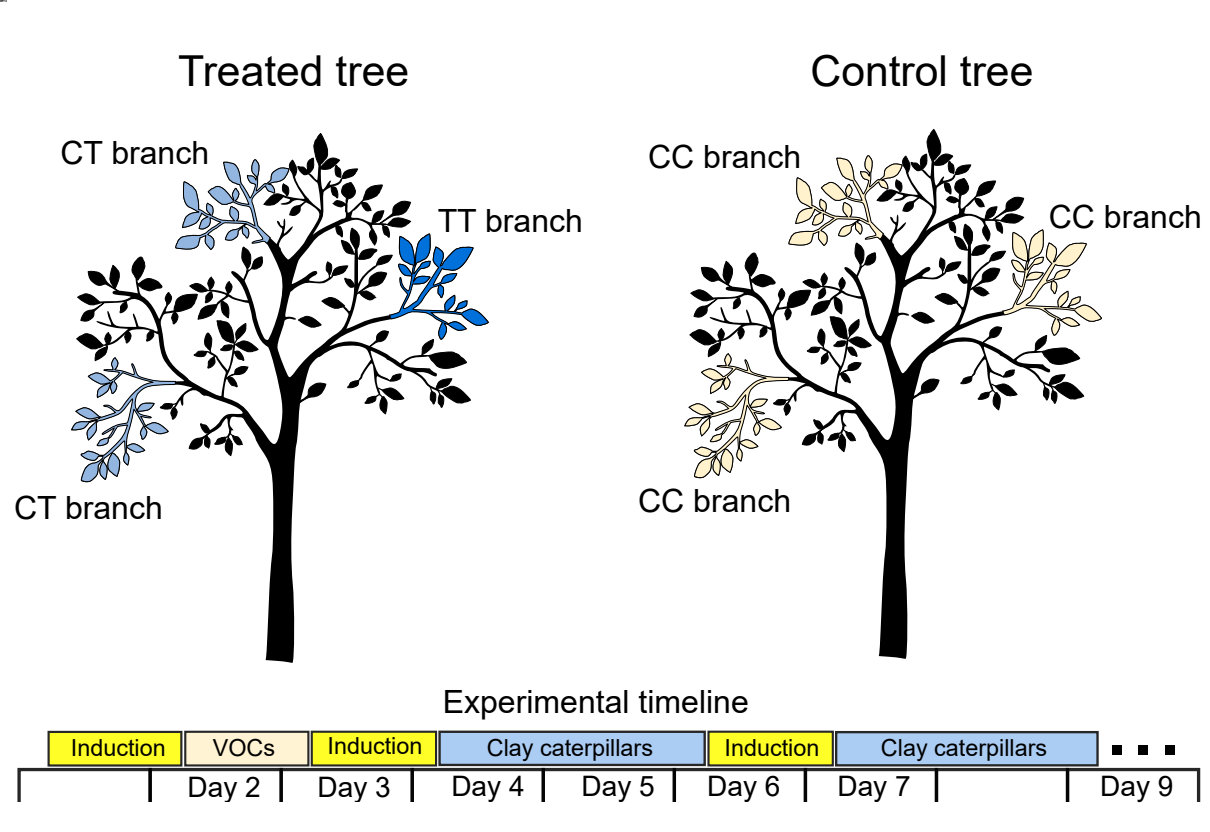
789 VOCs showing individual significant response to the treatment when analysed with LMEs.
790 Other VOCs are in grey.

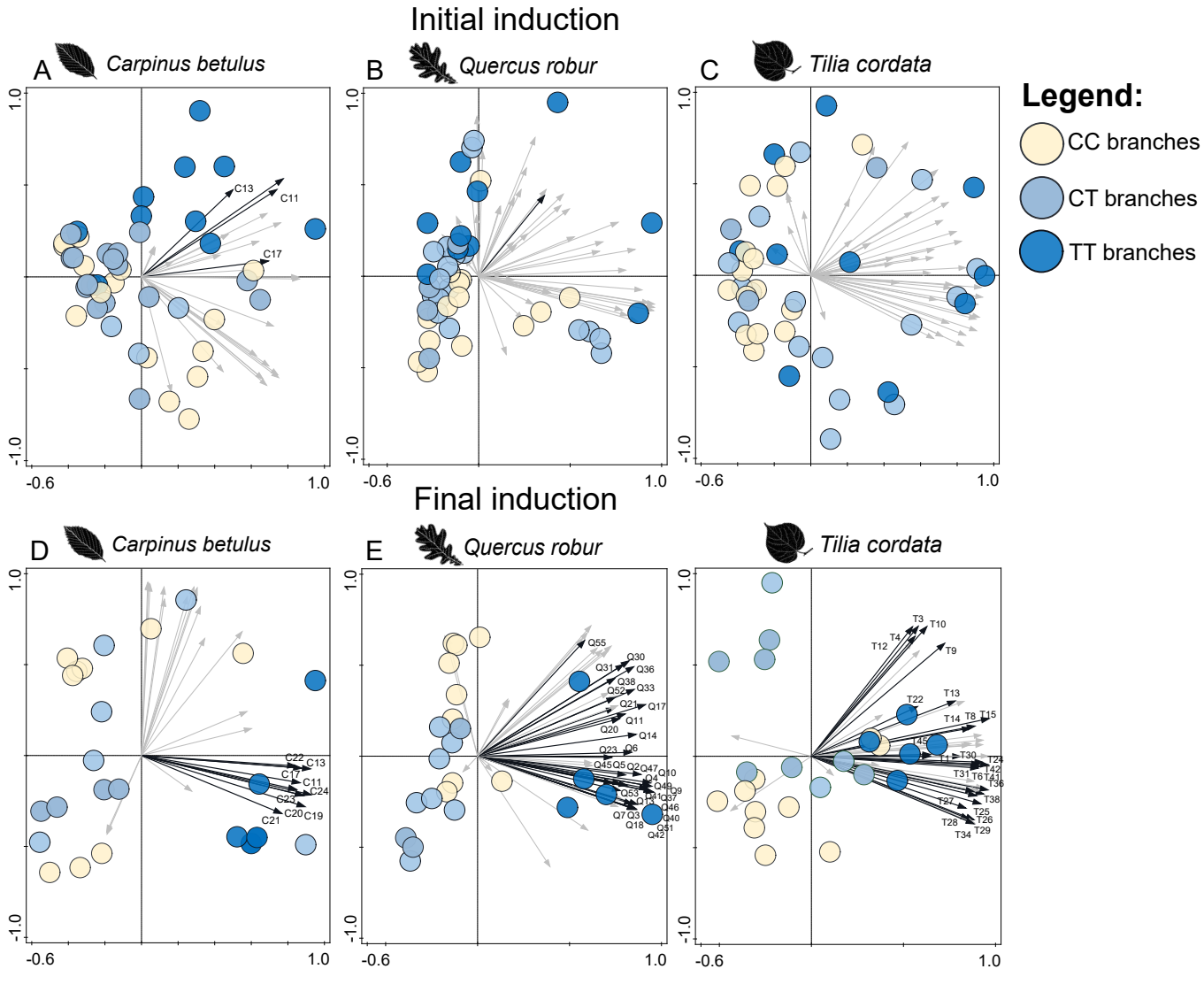
791 Figure 3. Increase in volatile organic compound (VOC) emissions in TT branches (TT – treated
792 branches on treated trees) in comparison to CC branches (CC – control branches on control
793 trees) in *Carpinus betulus* (A, D), *Quercus robur* (B, E), and *Tilia cordata* (C, F) after the initial
794 (upper row) and final induction (lower row). The bars show the number of VOCs from the
795 recorded groups that increased 1-10x, 11-100x, 101-1,000x, 1,001-10,000x or appeared in the
796 samples from TT branches only (marked as "New"). Only the compounds showing statistically
797 significant localized or systemic induction are shown (Table S2).

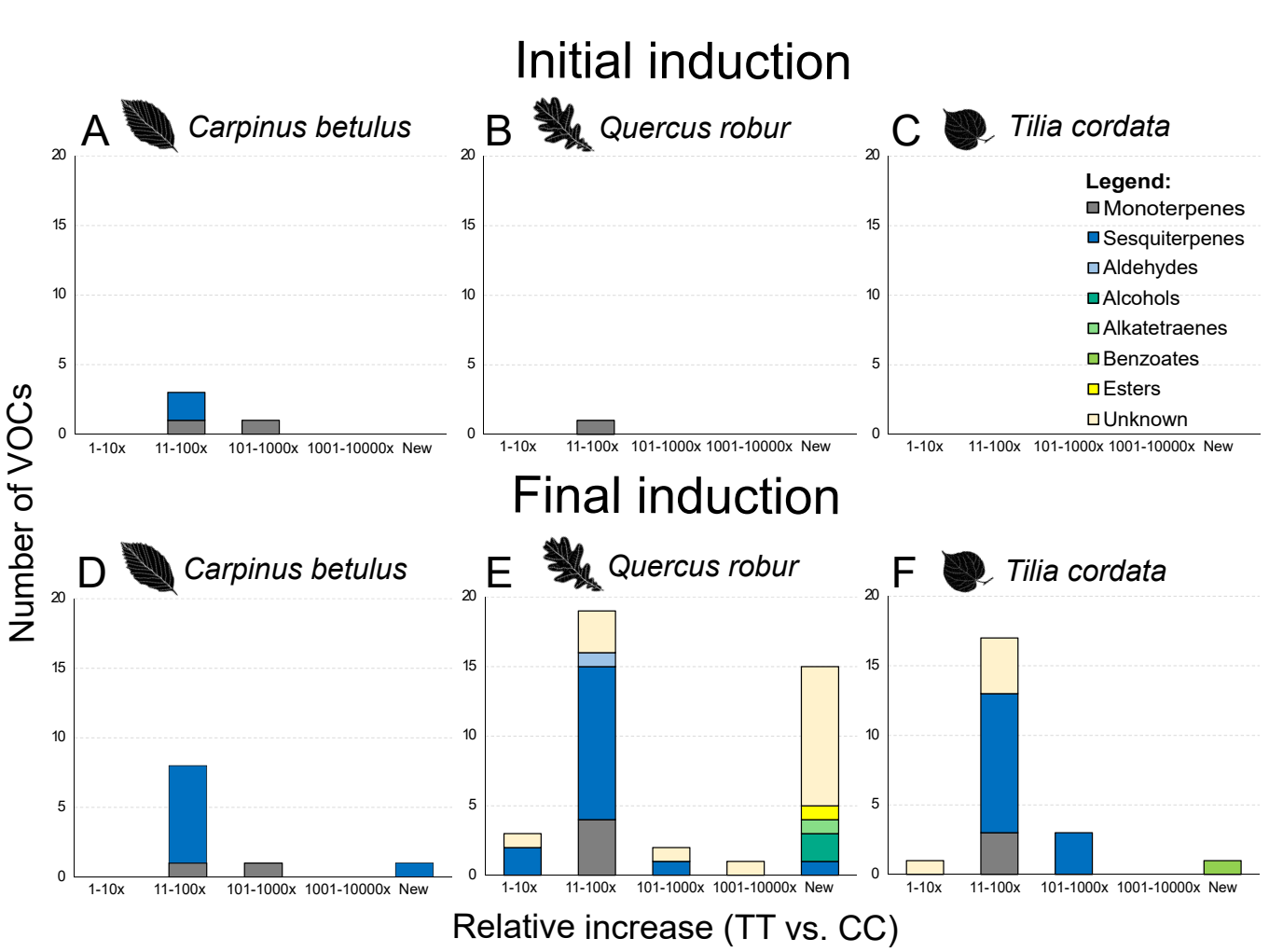
798 Figure 4. Average number of predation events on treated and control tree individuals. There
799 were more clay caterpillars predated on trees treated with methyl jasmonate ($F_1 = 5.04$, $p = 0.030$). The graph shows results combined for all three tree species studied. The boxes show
801 the first to third quartile with the medians as horizontal lines, the whiskers show range.
802 Significant differences are marked with asterisks.

803 Figure 5. Preference of *Operophtera brumata* caterpillars for leaves from *Carpinus betulus* (A)
804 and *Quercus robur* (B) in 'tree and branch comparisons'. In 'branch comparisons', each larva
805 was offered a disc from a TT branch (TT – treated branches on treated trees) and a CT branch
806 (CT – control branches on treated trees) from the same treated tree. In 'tree comparisons', each
807 larva was offered with a disc from a TT branch and a CC branch (CC – control branches on
808 control trees). In *C. betulus*, the caterpillars preferred the discs from CC branches in 'tree
809 comparison' ($\chi^2(1) = 4.22$, $p = 0.0401$) and the discs from CT branches in 'branch comparison'
810 ($\chi^2(1) = 6.36$, $p = 0.0117$). In the case of *Q. robur*, the caterpillars preferred the discs from CC
811 branches in 'tree comparison' ($\chi^2(1) = 5.93$, $p = 0.0149$) whereas they did not differentiate
812 between the leaf discs in 'branch comparison' ($\chi^2(1) = 1.14$, $p = 0.2855$). The boxes show the

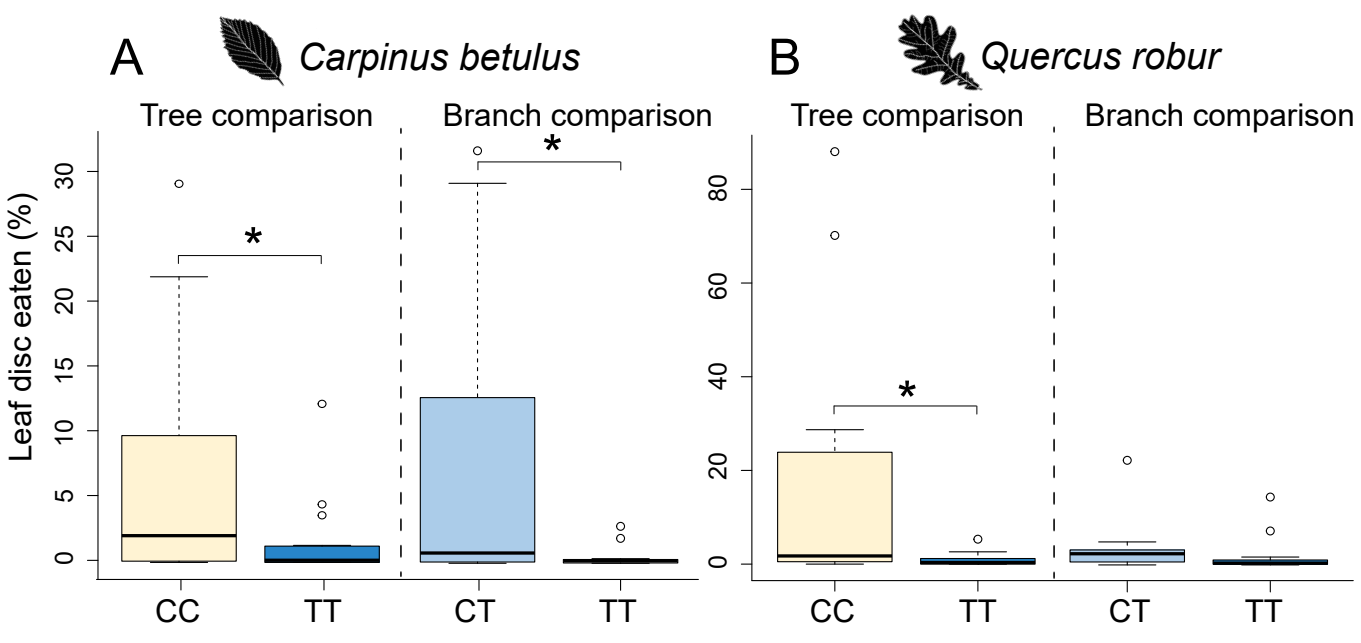
813 first to third quartile with the medians as horizontal lines, the whiskers show range. Significant
814 differences are marked with asterisks.
815


Treated tree
CT branch
TT branch
CT branch
Control tree
CC branch
CC branch
CC branch
Experimental timeline
Induction
VOCs
Induction
Clay caterpillars
Induction
Clay caterpillars
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 9


Initial induction
A
Carpinus betulus
B
Quercus robur
C
Tilia cordata
Legend:
CC branches
CT branches
TT branches
Final induction
D
Carpinus betulus
E
Quercus robur
Tilia cordata
1.0
-1.0
-0.6
1.0


Initial induction
A
Carpinus betulus
B
Quercus robur
C
Tilia cordata
Legend:
Monoterpenes
Sesquiterpenes
Aldehydes
Alcohols
Alkatetraenes
Benzoates
Esters
Unknown
Final induction
D
Carpinus betulus
E
Quercus robur
F
Tilia cordata
Number of VOCs
0
5
10
15
20
1-10x
11-100x
101-1000x
1001-10000x
New
Relative increase (TT vs. CC)

Predation events
0
2
4
6
8
*
Control trees
Treated trees


A
Carpinus betulus
Tree comparison
Branch comparison
Leaf disc eaten (%)
0
5
10
15
20
25
30
*
*
CC
TT
CT
TT
B
Quercus robur
Tree comparison
Branch comparison
0
20
40
60
80
*
CC
TT
CT
TT

BRANCH-LOCALIZED INDUCTION PROMOTES EFFICACY OF VOLATILE DEFENCES AND HERBIVORE PREDATION IN TREES

MARTIN VOLF, ALEXANDER WEINHOLD, CARLO L. SEIFERT, TEREZA HOLICOVÁ, HENRIETTE UTHE, ERIKA ALANDER, RONNY RICHTER, JUHA-PEKKA SALMINEN, CHRISTIAN WIRTH, NICOLE M. van DAM

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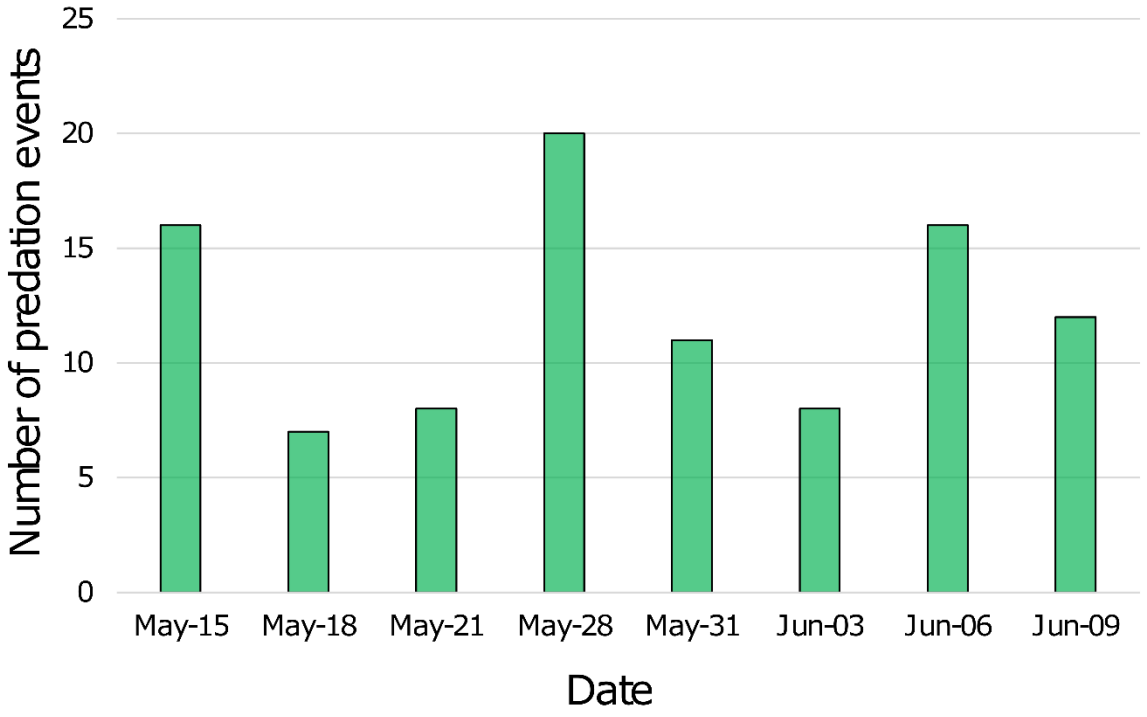

Figure S1. Number of predation events recorded on individual dates. Predation rates were measured using 225 clay caterpillars exposed on the studies trees for 48 hours.

BRANCH-LOCALIZED INDUCTION PROMOTES EFFICACY OF VOLATILE DEFENCES AND HERBIVORE PREDATION IN TREES

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Table S1. Volatiles detected in CC, CT, and TT branches in *Carpinus betulus*, *Quercus robur*, and *Tilia cordata*. The values show peak areas ± S.D. for individual volatiles sampled during the first (initial) and second (final) volatile sampling. We aimed at identifying the compounds showing a significant response to our treatment (in bold, Table S2). Measured retention indexes (RI) and retention indexes based on literature (RI Lit) are shown in the respective columns. Tentative names are marked with question marks.

Carpinus					Initial Sampling			Final Sampling		
Volatile	**Class**	**Name**	**RI**	**RI Lit**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
C1	unknown	unknown			210.8±142.2	503.6±596	585±865.5	328.5±363.4	823.5±654.8	1960.4±958.5
C2	unknown	unknown			2501.7±2639.5	1480.6±1649.4	1648±2980.3	21842.8±24408.3	23095.9±56949.3	7352.2±10727.6
C3	unknown	unknown			2163.6±2597.8	851.9±1002.5	1050±1886.5	25679.7±28983.3	25546.5±64005.4	7749.7±12277.9
C4	unknown	unknown			2162.3±2596.5	852.1±997.4	1048.3±1887.4	25393±28732.1	25359.6±63562	7727.8±12233.2
C5	unknown	unknown			0	75.4±250	195.8±619.2	255.5±499	179.3±567	219.7±491.3
C6	unknown	unknown			83±112.9	40.6±110.6	45.4±143.7	411.8±421.2	371.6±917.5	137.7±308
C7	unknown	unknown			263.6±315.5	109.4±212.2	139.6±365.9	1752±1933.7	2029.3±5035	686.7±1338.1
C8	unknown	unknown			438.4±452	471±435.7	431.3±703.4	2179.9±2348.4	2047.9±4564.8	704.3±985.3
C9	unknown	unknown			2983.1±5115.7	2640.8±4010.8	2297.2±3198.9	2621.2±2604.8	2337.1±5296.6	980.9±1675.5
C10	unknown	unknown			996.8±1078.4	822.9±1464.9	3239±8391.6	5223.1±5447.3	8864.7±15412.1	7028.5±8284.7
C11	**monoterpene**	**p- cymene**	1288	1282 (1)	146.4±422	237.3±686.4	16733.1±32315.9	48.6±145.7	2922.5±9241.8	4339.5±4222.9
C12	unknown	unknown			142.1±176.2	68.1±138.6	76.6±172.1	846.4±909	864.9±2049.3	307.4±586.9
C13	**oxy monoterpene**	**1,7-octadien-3-one, 2-methyl-6-methylene**	1319	1345 (2)	1247.8±2414.8	2600.2±3308.3	88913.7±123363.9	481.3±1023.1	69253.3±216796.5	76899.4±60840.1
C14	unknown	unknown			0	0	0	1006.6±1172.8	1290.1±3507.7	392.5±877.8
C15	unknown	unknown			133.9±306.1	40.2±179.7	0	989.9±965	1224.2±3122.7	535.2±1196.8
C16	unknown	unknown			0	0	125.1±395.7	412.5±610.4	2193.5±2741.2	5617.5±6226.7
C17	**sesquiterpene**	**caryophyllene**	1631	1608 (1)	426.1±553.5	281.8±353	5042.4±7970	1264.1±3143.7	16517.7±51210.8	50799.4±50849.5
C18	unknown	unknown			0	0	196.6±621.6	0	0	270.5±604.8
C19	**sesquiterpene**	**humulene**	1704	1710 (3)	56.4±165.2	48.6±117.6	3072.3±5452.2	935.4±2720	12381.1±39068	22941.3±27591.4
C20	**sesquiterpene**	**γ-muurolene**	1716	1684 (1)	12.2±25.4	20.7±41.1	126±270.3	23.7±63.8	197.6±606.3	714.1±604.9
C21	**sesquiterpene**	**farnesene type?**	1732	1674(4)	1536.9±3067.7	711.4±2889.2	1395.7±3826.4	0	163±515.5	924.2±762.7

Carpinus					Initial Sampling			Final Sampling		
Volatile	**Class**	**Name**	**RI**	**RI Lit**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
C22	**sesquiterpene**	**muurolene type?**	1741	1684 (4)	8.8±34.3	2.5±11.4	168.4±502.9	74.5±209.3	591.8±1706.6	2024.1±598.8
C23	**sesquiterpene**	**α-farnesene**	1757	1727 (5)	3786.3±7312.4	1710.6±6223.1	5818.7±15385.5	162.5±277.7	1384.8±4022.5	13079.1±14803.6
C24	**sesquiterpene**	**unknown**			61.1±98.1	36.9±72.4	348.9±563.1	87.2±204.2	335.2±1001.7	1460.4±1886
C25	**sesquiterpene**	**unknown**			64.3±96.3	39.2±71.9	348.4±563.4	87.2±204.2	335.4±1002.3	1467.6±1884.4
C26	unknown	unknown			869.3±889.6	1521.8±1496	1952.5±1620.1	845.8±475.9	860.6±575.1	1028.1±1159.7
C27	unknown	unknown			2879.2±2953.2	5197±6186	5699.4±4425.6	2081.6±1473.9	2452.1±2062	2847.7±3223.3
Quercus					Initial Sampling			Final Sampling		
Volatile	**Class**	**Name**	**RI**	**RI Lit**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
Q1	unknown	unknown			74.9±168.7	58±117.2	66.6±107.6	38.1±76	87±99.8	810.9±927.8
Q2	**monoterpene**	**β-pinene**	1175	1124 (1)	24.6±68.9	104.2±211.4	191.8±244.1	138.1±184.6	28.8±65.4	3147.9±3899
Q3	**aldehyde**	**2-butenal, 3-methyl- / prenal / 3-methylcrotonaldehyd**	1218	1221 (6)	0	0	143.1±294.7	205±577.7	24.4±77.1	2199.4±2364.3
Q4	**monoterpene**	**cis-β-ocimene**	1246	1245 (1)	112.3±208.5	67.3±142.5	3305.9±5812.3	3979.3±9935.8	489.6±1163.7	72535.2±89413.4
Q5	**monoterpene**	**trans-β-ocimene**	1267	1250 (7)	1350.2±1522.4	1530.2±1426.4	32055.6±54069.4	34247.1±80213.7	5113.8±12081.6	943439.1±816041.4
Q6	**unknown**	**unknown**			469.5±637.9	690.6±1481.4	19195.4±36056	6886.7±9658.1	1266.6±864.5	291037.6±142115.4
Q7	**unknown**	**unknown**			0	0	141.1±446.1	0	0	18364.4±22244.1
Q8	**unknown**	**unknown**			201.1±778.9	51.9±232	145.9±461.5	533.5±1600.5	0	5093.3±4508
Q9	**unknown**	**unknown**			0	0	21.7±47.3	46.4±139.3	0	1033.9±1182.8
Q10	**alkatetraene**	**2,6-dimethyl-1,3,5,7-octatetraene, E,E- / cosmene**	1464	1460 (8)	0	0	0	0	0	20740.1±23500.9
Q11	**sesquiterpene**	**α-cubebene**	1480	1463 (1)	117.1±254.1	315.5±686.6	669.5±1352.6	1698±1977.7	242.7±344.6	51140.1±45583.7
Q12	**unknown**	**unknown**			0	0	79.7±206.6	0	0	2498±5022.8
Q13	**unknown**	**unknown**			0	0	95.4±301.7	490.8±1408.6	79.6±251.8	27774.3±40000.6
Q14	**sesquiterpene**	**unknown**			0	0	0	143.4±349.6	0	4859.3±3471.6
Q15	**sesquiterpene**	**unknown**			968.6±1038	682.9±753.4	1352±1522.3	14330.4±11080.6	3586.5±4668.1	23441.8±16752.4
Q16	**sesquiterpene**	**β-bourbene**	1550	1546 (9)	380.8±1220.9	2846.7±5967	6202.4±14431.7	16742.8±24555.1	3597.6±7167.7	332459.4±256996.1
Q17	**sesquiterpene**	**unknown**			68.7±140.8	172.4±311.2	461.2±878.7	1582.2±1759.7	579.3±720.6	51719.4±40254.3
Q18	**alcohol**	**unknown**			0	0	68.5±216.6	0	0	28196.4±41185.4
Q19	unknown	unknown			529.1±787.8	581.8±996.5	524.6±988.1	744.2±1240.9	454.3±444.2	9993.8±13001.3
Q20	**sesquiterpene**	**unknown**			49.9±146.7	221.9±458.6	416.6±907.2	1002.5±1186.8	367.3±828.4	33664.9±25419
Q21	**sesquiterpene**	**unknown**			156.6±460.7	569.9±1182.7	1197±2683.4	2441±2881.6	722.7±1859.3	76115±53881.9
Q22	**sesquiterpene**	**caryophyllene**	1632	1608 (1)	937.2±1657.7	1363.6±1363.1	2332.2±2242.4	13639±12293.1	5651±5383.3	64938.5±65477.3
Q23	**sesquiterpene**	**unknown**			0	0	0	0	0	7796.4±8862.2
Q24	unknown	unknown			69.3±268.6	35.5±158.9	0	0	0	3261.5±7292.9

Quercus					Initial Sampling			Final Sampling		
Volatile	Class	Name	RI	RI Lit	CC	CT	TT	CC	CT	TT
Q26	**sesquiterpene**	**unknown**			0	81.4±199.1	230.9±555.7	896.5±976.4	121.2±221.3	18736.5±16155
Q27	unknown	unknown			0	0	12.2±38.6	547.2±554.5	166.5±264.2	221.6±495.4
Q28	unknown	unknown			0	0	0	212.9±277.4	0	0
Q29	unknown	unknown			0	0	0	39.3±117.8	0	0
Q30	**sesquiterpene**	**humulene**	1704	1710 (3)	41.9±94.6	129.2±282.8	429.4±898.5	2295.4±2267.7	485.1±595.1	52657.9±45080.3
Q31	**sesquiterpene**	**γ-muurolene**	1716	1684 (1)	117.9±174.7	256.3±435.5	644.5±1110.6	5956.3±5282.4	1200.1±1444.4	52499.9±41137.8
Q32	unknown	unknown			554.8±1807.1	1602.9±4945.1	1958.7±3020.4	1330.2±1530.9	3662.3±9542.2	41706.8±81329.6
Q33	**sesquiterpene**	**unknown**			45.8±98.3	176.3±467.8	779.1±1590	2941.1±3810.5	313.5±511.7	490149.8±444970.7
Q34	**sesquiterpene**	**α-farnesene**	1760	1727 (5)	3253.7±9966	8286±25599.1	7463.5±11164.9	20351.2±16205.9	16024.6±35977.2	400577±722965.4
Q35	**sesquiterpene**	**unknown**			695±641.6	1005.5±934.8	2791.5±4338.4	12695±10858.3	4211.2±5782.1	56124.6±44202.4
Q36	**sesquiterpene**	**unknown**			151.2±179.8	296.3±522.4	627.6±1217.4	4020.4±3241.9	1099.9±1318.3	52699.5±42802.6
Q37	**unknown**	**unknown**			0	0	173.4±548.3	42.8±128.5	0	55273.5±89001.5
Q38	**sesquiterpene**	**unknown**			32.8±104.2	69.1±258.5	171.3±309	592.7±676.9	58.1±108.7	13060.8±11118.4
Q39	**sesquiterpene**	**unknown**			0	10.1±45.1	159.7±294.5	1389.2±1009.8	381±462.9	12968.3±11734.6
Q40	**unknown**	**unknown**			0	0	42.4±134.2	0	0	11313.3±17251.8
Q41	**ester**	**acetic acid, 2-phenylethyl ester**	1835	1821 (10)	0	0	0	0	0	7417.1±14723.5
Q42	**unknown**	**unknown**			0	0	0	0	0	4874.9±8240.1
Q43	**unknown**	**unknown**			136.9±157.7	348.6±710.4	229.2±212.8	2203.9±2168	1049.9±1853.1	6735.8±5955
Q44	unknown	unknown			68.7±266.2	252.6±824.9	153.4±332.8	55.8±167.3	389.2±1230.9	3557.8±7365.7
Q45	**unknown**	**unknown**			43.4±168.1	191.6±660.9	126.3±271.8	18.2±54.6	351.8±946.8	4206.6±7869.4
Q46	**unknown**	**unknown**			0	31.5±140.8	0	0	0	7062.1±9557.8
Q47	**alcohol**	**phenylethyl alcohol**	1927	1931 (11)	109.3±152.9	353.2±445.1	355.7±424.3	0	143.5±402.5	8679.3±16490.6
Q48	**sesquiterpene**	**α-calacorene**	1942	1948 (12)	40.5±64.9	114.7±211.1	196.6±259.3	1602.1±1082.2	523.6±644	2820.9±1988.9
Q49	**unknown**	**unknown**			0	30±134	42.1±133.3	0	0	4448.6±7938.4
Q51	**unknown**	**unknown**			0	0	0	0	0	4765.7±8523
Q52	**sesquiterpene alcohol**	**cyclohexene, 6-(2-butenyl)-1,5,5-trimethyl-, (E)-**	2042	2027 (13)	5.2±20.1	156±331.8	378±892.9	372.7±434.2	118.9±219.2	5949.2±4883.6
Q53	**unknown**	**unknown**			0	0	28.5±90.3	0	408.4±1132.2	58114.6±113143.7
Q54	unknown	unknown			12.2±38.4	23.5±69.7	45.9±113.9	1190.5±885.4	306.9±334.6	2309.4±1418.1
Q55	**hydroxy sesquiterpene**	**α-cadinol**	2204	2229 (14)	0	0	0	506.8±464.5	65.8±146.6	1017.7±683.4
Q56	unknown	unknown			0	2.2±9.7	8.5±26.9	2105.2±2344	629.3±728.9	766.5±564.5
Q57	unknown	unknown			0	10.6±47.3	0	873.2±799	159.6±227.7	4293.3±2848.5
Q58	**unknown**	**unknown**			0	0	0	0	0	3350.6±4031.4
Q59	**unknown**	**unknown**			0	0	0	0	0	775.2±992.5

Quercus					Initial Sampling			Final Sampling		
Volatile	**Class**	**Name**	**RI**	**RI Lit**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
Q60	unknown	unknown			1078.5±796.5	1376.3±832.6	1291.6±1044	820.5±584.9	510.3±299.8	554.4±153.2
Q61	unknown	unknown			351.2±1116.1	84±207.2	180.2±508.9	19.8±59.3	0	0
Tilia					Initial Sampling			Final Sampling		
Volatile	**Class**	**Name**	**RI**	**RI Lit**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
T1	**unknown**	**unknown**			324.4±217.4	496.5±257.9	726.3±846.4	546.3±404.2	466.9±260	4406.6±4693.8
T2	unknown	unknown			0	0	23.3±73.7	123.2±252.3	0	262.1±315.4
T3	**monoterpene**	**α-pinene**	1041	1034 (1)	1516.9±1413.4	310.6±237.8	416.6±342.9	5155.7±8594.7	1123.7±1804.7	9212.5±6020.3
T4	**monoterpene**	**campherene**	1084	1077 (1)	3806.4±3926.5	402.1±578.9	555.4±709.6	11430.9±19525.6	1668.6±3346.1	10197.6±8848.9
T5	unknown	unknown			618.7±1706.3	1277±3139.6	2388±3418.9	2216.1±4180.7	1510.7±2823.3	54667.7±87221.8
T6	**unknown**	**unknown**			17.7±68.7	36.4±93.7	85.2±133.7	68.7±152.9	83.2±172	1524.5±1100.7
T7	unknown	unknown			1308.9±1295	200.7±216.2	359±408.6	5004.5±8763.4	1070.8±1639.3	5935.2±3549.4
T8	**unknown**	**unknown**			0	5.1±22.3	0	53.8±75.7	156.4±336.3	3581±5005
T9	**monoterpene**	**β-myrcene**	1173	1168 (1)	68.3±70.2	108.2±64.5	109.6±57.4	498.2±569.1	179.7±380.8	3422.8±2315.6
T10	**monoterpene**	**1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)- / α-terpinene**	1197	1178 (15)	17.1±35.7	0	0	125.6±192	27±57	351.1±336.2
T11	unknown	unknown	1218	1212 (16)	2133.9±1567.3	1816.2±763.1	1457.6±705.2	1198±1092.1	980.6±625.2	4766.8±5957.4
T12	**monoterpene**	**D-limonene**	1228	1224 (16)	1303.5±1072.9	240.5±208.5	312±293.2	5491±9229	1124±2094.9	6666.5±4458.1
T13	**oxy monoterpene**	**eucalyptol**	1244	1245 (1)	540.5±746.9	732.1±844.3	953.9±1174.8	4792.9±4435.2	3464.1±4649	105923.8±160489.7
T14	**monoterpene**	**cis-β-ocimene**	1262	1250 (7)	196.9±428.1	309.7±341.5	310±299.9	351.4±621.2	406.5±821.1	6359.7±4038.6
T15	**monoterpene**	**trans -β-ocimene**			3860.7±7327.8	10713.5±15580	17123.6±26896.2	20378.8±32997.4	24794±51042.5	616374.3±404896.9
T16	unknown	unknown			95.7±370.7	960.7±1611.8	1017.7±1056.4	584.4±984	3939.6±9537.3	4360.5±2526.5
T17	unknown	unknown			48.5±74.9	0	7±22.2	301.6±511.9	63.7±137.4	1054.7±935.5
T18	unknown	unknown			417.8±1337	4817±8225.9	10846.9±24408.6	15967.1±33603.4	148577.1±346961.7	403690.3±229575.2
T20	unknown	unknown			0	0	45.1±142.6	34±77.9	23.7±50.1	1171.3±1203.4
T19	unknown	unknown			5875.6±3824.9	12552.5±10136	12692.1±11917.3	3490.2±5447.6	7266.2±9087.1	9741.1±16174
T21	unknown	unknown			36.4±141	553.5±1550.7	442.8±968.9	133±398.9	150.5±475.8	0
T22	**unknown**	**unknown**			0	0	317.1±735.8	308±620.6	0	3531.6±3329
T23	unknown	unknown			0	476.6±1123	1193.9±2130.9	1401±2326.4	2293.2±4243.9	43735.4±37334
T24	**sesquiterpene**	**unknown**			0	76.1±216.9	225.6±420	296.2±437.2	416.3±782.3	8659.1±7743.5
T25	**sesquiterpene**	**unknown**			62.7±93.7	423.8±754.7	846.6±1330.8	1038±1371.8	1663.1±2528.8	31456.9±29051.5
T26	**sesquiterpene**	**(-)-β-bourbonene**	1551	1546 (9)	954.5±1105.8	4909.7±9750.1	5014.9±7077.9	3537.5±5426.5	9412.5±14751.1	259910.9±297527.9
T27	**sesquiterpene**	**unknown**			5.8±15.4	472.5±1088	987.2±1703	1738.8±2939.6	3443.4±6627.8	103100.1±110715.6
T28	**sesquiterpene**	**unknown**			45.1±97.5	298.3±582.2	565.6±930.7	709.5±1192.5	1414.8±2581.5	42648.6±44057.8

Tilia					Initial Sampling			Final Sampling		
Volatile	Class	Name	RI	RI Lit	CC	CT	TT	CC	CT	TT
T29	**sesquiterpene**	**unknown**			58.4±75	873.2±1912.3	1824.3±3201.3	2463.3±4108.9	4417.3±8297.5	125185.2±127748.7
T30	**sesquiterpene**	**caryophyllene**	1631	1608 (1)	2722.8±4064.8	2514±2424.9	2868.3±3228.5	624.1±626.5	2752.9±2803.6	68357.4±42110.2
T31	**sesquiterpene**	**β-copaene?**			0	28.3±123.3	66±165.5	278±517.7	696.8±1472.4	25581.8±32201
T32	unknown	unknown			0	238.8±600.1	706.2±1309.7	652.8±1111.3	1213.4±2301.6	36041.3±35025.7
T33	unknown	unknown			2.2±8.6	583.4±1353.9	1502.6±2676.9	1720.5±2884.2	3278.6±6134.7	93845.4±94611.6
T34	**sesquiterpene**	**γ-muurolene**	1716	1684 (1)	0	635.2±1469.3	1649.9±2949.3	1990.2±3190.3	3715±6875.3	96037.2±91859.9
T35	unknown	unknown			1204.8±1670.4	3270.3±4535.6	10256.6±17038.5	3774.3±9087.4	4692±10611.9	75570.3±70431
T36	**sesquiterpene**	**unknown**			8.7±33.8	1250.2±2744.4	2548.8±4438.1	8528.2±15323.8	32229.6±70766.8	1170362.6±1217899.1
T37	unknown	unknown			5831.8±6792.9	30058.2±52637.2	112133.9±215190	74392.1±163045.1	103501.2±177189.7	1117743.6±865190.7
T38	**sesquiterpene**	**unknown**			126.8±86.8	1145.5±1776.5	2322.6±3374.5	2795.7±4038.1	5477.8±7803.9	110181.1±106614.9
T39	unknown	unknown			4.5±17.3	666±1573.5	1766.6±3121.8	2094.8±3240.9	3775.8±6918.5	105164.6±106100.9
T40	unknown	unknown			4.6±17.7	156.3±427.1	440.9±849	494.7±749.9	803.2±1550.5	21991.7±20487.6
T41	**sesquiterpene**	**unknown**			0	115.3±326.3	317±673.6	486.9±717.7	870.1±1673.8	29477.1±31264.8
T42	**sesquiterpene**	**unknown**			0	86±219.5	426.9±908.7	246.7±701.1	333.7±653.7	41879.3±53875.2
T43	unknown	unknown			79.3±153.3	265±403	1014±1963.1	352.9±918.6	451.2±921.3	3335.5±1958.1
T44	unknown	unknown			14.8±57.5	151.1±214.6	299.2±438.2	171.8±382.8	309.8±546.7	1006.7±953.5
T45	**benzoate**	**1-butanol, 3-methyl-, benzoate**	1934	1929(17)	0	0	0	0	64.1±138.5	32258.4±46640.3
T46	**unknown**	**unknown**			871.1±1961.3	673.3±1554.5	2180.4±4247.7	1801.5±4692.5	1768.3±3611.5	65600.1±72277.0

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BRANCH-LOCALIZED INDUCTION PROMOTES EFFICACY OF VOLATILE DEFENCES AND HERBIVORE PREDATION IN TREES

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Table S2. Correlation of individual volatiles to the treatment in CC, CT, and TT branches in *Carpinus betulus*, *Quercus robur*, and *Tilia cordata* in the first (initial) and second (final) volatile sampling as analyzed by LMEs. We defined locally induced compounds as those significantly higher in TT branches in comparison to other branches while showing no significant difference between CC and CT branches. We defined systemically induced compounds as those significantly lower in CC branches in comparison to both TT and CT branches. Other trends we recorded are reported as "Other". Only the volatiles that showed some significant response are shown. VOCs that appeared only after the induction treatment have their trends in bold and marked with asterisks.

Carpinus			**Initial Sampling**			**Final Sampling**		
Volatile	**Class**	**name**	**$\chi^2(2)$**	**p**	**trend**	**$\chi^2(2)$**	**p**	**trend**
C11	monoterpene	p- cymene	21.5	<0.0001	Localized induction	12.9	0.0016	Localized induction
C13	oxy monoterpene	1,7-octadien-3-one, 2-methyl-6-methylene	20.1	<0.0001	Localized induction	13.1	0.0014	Localized induction
C17	sesquiterpene	caryophyllene	14.3	0.0008	Localized induction	12.9	0.0016	Localized induction
C19	sesquiterpene	humulene	23.6	<0.0001	Localized induction	17.2	0.0002	Localized induction
C20	sesquiterpene	γ-muurolene	-	-	n.s.	19.3	<0.0001	Localized induction
C21	sesquiterpene	farnesene type?	-	-	n.s.	19.0	<0.0001	Localized induction
C22	sesquiterpene	muurolene type?	-	-	n.s.	16.9	0.0002	Localized induction
C23	sesquiterpene	Alpha farnesene?	-	-	n.s.	14.9	0.0006	Localized induction
C24	sesquiterpene	unknown	-	-	n.s.	13.1	0.0014	Localized induction
C25	sesquiterpene	unknown	-	-	n.s.	13.1	0.0014	**Localized induction***
Quercus			**Initial Sampling**			**Final Sampling**		
Volatile	**Class**	**name**	**$\chi^2(2)$**	**p**	**trend**	**$\chi^2(2)$**	**p**	**trend**
Q2	monoterpene	β-pinene	-	-	n.s.	20.9	<0.0001	Localized induction
Q3	aldehyde	2-butenal, 3-methyl- / prenal / 3-methylcrotonaldehyd	-	-	n.s.	24.6	<0.0001	Localized induction
Q4	monoterpene	cis-β-ocimene	-	-	n.s.	16.6	0.0002	Localized induction
Q5	monoterpene	trans-β-ocimene	16.9	0.0002	Localized induction	30.8	<0.0001	Localized induction
Q6	unknown	unknown	-	-	n.s.	25.0	<0.0001	Localized induction
Q7	unknown	unknown	-	-	n.s.	33.0	<0.0001	**Localized induction***
Q8	unknown	unknown	-	-	n.s.	20.6	<0.0001	Localized induction
Q9	unknown	unknown	-	-	n.s.	38.3	<0.0001	Localized induction
Q10	alkatetraene	2,6-dimethyl-1,3,5,7-octatetraene, E,E- / cosmene	-	-	n.s.	99.8	<0.0001	**Localized induction***
Q11	sesquiterpene	α-cubebene	-	-	n.s.	32.4	<0.0001	Localized induction

Quercus			Initial Sampling			Final Sampling		
Volatile	**Class**	**name**	**$\chi^2(2)$**	**p**	**trend**	**$\chi^2(2)$**	**p**	**trend**
Q12	unknown	unknown	-	-	n.s.	17.4	0.0002	**Localized induction***
Q13	unknown	unknown	-	-	n.s.	27.1	<0.0001	Localized induction
Q14	sesquiterpene	unknown	-	-	n.s.	38.7	<0.0001	Localized induction
Q15	sesquiterpene	unknown	-	-	n.s.	14.9	0.0005	Other
Q16	sesquiterpene	β-bourbene	-	-	n.s.	18.4	0.0001	Other
Q17	sesquiterpene	unknown	-	-	n.s.	35.3	<0.0001	Localized induction
Q18	alcohol	unknown	-	-	n.s.	32.9	<0.0001	**Localized induction***
Q20	sesquiterpene	unknown	-	-	n.s.	23.7	<0.0001	Localized induction
Q21	sesquiterpene	unknown	-	-	n.s.	26.6	<0.0001	Localized induction
Q22	sesquiterpene	caryophyllene	-	-	n.s.	15.3	0.0004	Other
Q23	sesquiterpene	unknown	-	-	n.s.	18.6	<0.0001	**Localized induction***
Q26	sesquiterpene	unknown	-	-	n.s.	17.6	0.0002	Localized induction
Q30	sesquiterpene	humulene	-	-	n.s.	21.4	<0.0001	Localized induction
Q31	sesquiterpene	γ-muurolene	-	-	n.s.	25.1	<0.0001	Localized induction
Q33	sesquiterpene	unknown	-	-	n.s.	32.9	<0.0001	Localized induction
Q34	sesquiterpene	α-farnesene	-	-	n.s.	15.7	0.0004	Localized induction
Q35	sesquiterpene	unknown	-	-	n.s.	16.8	0.0002	Other
Q36	sesquiterpene	unknown	-	-	n.s.	25.9	<0.0001	Localized induction
Q37	unknown	unknown	-	-	n.s.	55.3	<0.0001	Localized induction
Q38	sesquiterpene	unknown	-	-	n.s.	19.9	<0.0001	Localized induction
Q39	sesquiterpene	unknown	-	-	n.s.	16.1	0.0003	Other
Q40	unknown	unknown	-	-	n.s.	79.1	<0.0001	**Localized induction***
Q41	ester	acetic acid, 2-phenylethyl ester	-	-	n.s.	59.4	<0.0001	**Localized induction***
Q42	unknown	unknown	-	-	n.s.	31.7	<0.0001	**Localized induction***
Q43	unknown	unknown	-	-	n.s.	13.7	0.0011	Other
Q45	unknown	unknown	-	-	n.s.	19.2	<0.0001	Localized induction
Q46	unknown	unknown	-	-	n.s.	32.6	<0.0001	**Localized induction***
Q47	alcohol	phenylethyl alcohol	-	-	n.s.	18.7	<0.0001	**Localized induction***
Q48	sesquiterpene	α-calacorene	-	-	n.s.	14.2	0.0008	Other
Q49	unknown	unknown	-	-	n.s.	67.2	<0.0001	**Localized induction***
Q51	unknown	unknown	-	-	n.s.	30.9	<0.0001	**Localized induction***
Q52	sesquiterpene alcohol	cyclohexene, 6-(2-butenyl)-1,5,5-trimethyl-, (E)-	-	-	n.s.	18.5	<0.0001	Localized induction
Q53	unknown	unknown	-	-	n.s.	28.7	<0.0001	**Systemic induction***
Q55	hydroxy sesquiterpene	α-cadinol	-	-	n.s.	19.6	<0.0001	Localized induction
Q58	unknown	unknown	-	-	n.s.	32.3	<0.0001	**Localized induction***
Q59	unknown	unknown	-	-	n.s.	17.9	0.0001	**Localized induction***

Tilia			Initial Sampling			Final Sampling		
Volatile	**Class**	**name**	**$\chi^2(2)$**	**p**	**trend**	**$\chi^2(2)$**	**p**	**trend**
T1	unknown	unknown	-	-	n.s.	19.3	<0.0001	Localized induction
T3	monoterpene	α-pinene	-	-	n.s.	16.4	0.0003	Other
T4	monoterpene	campherene	-	-	n.s.	20.0	<0.0001	Other
T6	unknown	unknown	-	-	n.s.	20.1	<0.0001	Localized induction
T8	unknown	unknown	-	-	n.s.	18.0	0.0001	Localized induction
T9	monoterpene	β-myrcene	-	-	n.s.	23.5	<0.0001	Other
T10	monoterpene	1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)- / α-terpinene	-	-	n.s.	16.4	0.0003	Other
T12	monoterpene	D-limonene	-	-	n.s.	14.4	0.0008	Other
T13	oxy monoterpene	eucalyptol	-	-	n.s.	19.8	<0.0001	Localized induction
T14	monoterpene	cis-β-ocimene	-	-	n.s.	16.9	0.0002	Localized induction
T15	monoterpene	trans -β-ocimene	-	-	n.s.	20.4	<0.0001	Localized induction
T22	unknown	unknown	-	-	n.s.	17.4	0.0002	Localized induction
T24	sesquiterpene	unknown	-	-	n.s.	13.8	0.0010	Localized induction
T25	sesquiterpene	unknown	-	-	n.s.	15.5	0.0004	Localized induction
T26	Sesquiterpene	(-)-β-bourbonene	-	-	n.s.	15.3	0.0005	Localized induction
T27	sesquiterpene	unknown	-	-	n.s.	13.7	0.0010	Localized induction
T28	sesquiterpene	unknown	-	-	n.s.	14.6	0.0007	Localized induction
T29	sesquiterpene	unknown	-	-	n.s.	14.8	0.0006	Localized induction
T30	sesquiterpene	caryophyllene	-	-	n.s.	15.8	0.0004	Systemic induction
T31	sesquiterpene	unknown	-	-	n.s.	16.6	0.0002	Localized induction
T34	sesquiterpene	γ-muurolene	-	-	n.s.	16.8	0.0002	Localized induction
T36	sesquiterpene	unknown	-	-	n.s.	15.9	0.0003	Localized induction
T38	sesquiterpene	unknown	-	-	n.s.	20.9	<0.0001	Localized induction
T41	sesquiterpene	unknown	-	-	n.s.	13.8	0.0010	Localized induction
T42	sesquiterpene	unknown	-	-	n.s.	19.4	<0.0001	Localized induction
T45	benzoate	1-butanol, 3-methyl-, benzoate	-	-	n.s.	19.3	<0.0001	**Localized induction***
T46	unknown	unknown	-	-	n.s.	17.4	0.0002	Localized induction

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Table S3. Polyphenol profiles, polyphenol activities, and protein content detected in CC branches, CT branches, and TT branches in *Carpinus betulus*, *Quercus robur*, and *Tilia cordata*. The values show average mg/g dry weight ± S.D for polyphenol contents, activities, and proteins, or average % ± S.D for PC/PA ratio.

	C. betulus			*Q. robur*			*T. cordata*		
	CC	**CT**	**TT**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
Total phenolics (mg/g)	93.5±26.4	95.9±14.7	90.3±14.3	50.4±18.7	50.8±15.6	59.7±15	19.9±13.4	22.9±15.5	23.7±23.7
Protein precipitation capacity (mg/g)	81.6±20.9	62.9±22.2	55.5±25.2	34.1±21.9	32.6±22.6	40.1±19.9	14.1±10.8	16.8±13.8	16.8±22.3
Polyphenol oxidative activity (mg/g)	23.1±5.6	22.8±5.3	20.7±4.2	21±8.8	20.3±6.9	22.9±5	1.3±3.0	1.2±1.9	1.0±2.3
Hydrolysable tannins									
Galloyl derivatives (mg/g)	13.7±2.6	11.8±3.9	11.4±4.2	1.1±0.5	1.6±0.8	1.7±0.5	0.0±0.0	0.0±0.0	0.1±0.1
HHDPs (mg/g)	40.1±7	33.9±11.6	35.6±4.7	10.8±3.8	12.7±5.4	12.4±2.7	0.0±0.0	0.0±0.0	0.1±0.2
Proanthocyanidins									
Procyanidin units of proanthocyanidis (PC) (mg/g)	1.6±1.3	1.6±2.4	1.4±1.8	2.5±3	2.7±2.3	5.4±4.5	11.1±9.0	15.3±12.3	15.9±18.6
Prodelphinidins of proanthocyanidis (PD) (mg/g)	1.1±1.4	1.1±2.2	0.9±1.7	0.2±0.3	0.2±0.3	0.6±0.6	0.6±1.4	0.7±1.2	0.7±2.0
PC/PA (%)	54.3±34.4	66.4±27.4	56.6±31.6	79.3±39.1	95.8±4.7	92.±4.0	97.1±5.7	97.3±4.7	97.5±3.8
Flavonol glycosides									
Kaempferol derivatives (mg/g)	0.1±0.1	0.1±0.1	0.1±0.1	2.7±0.5	3.1±0.8	2.9±0.8	0.2±0.1	0.2±0.0	0.2±0.0
Quercetin derivatives (mg/g)	0.7±0.2	1.1±0.7	0.8±0.6	1.1±0.3	1.3±0.5	1.1±0.3	0.2±0.3	0.1±0.1	0.1±0.2
Myricetin derivatives mg/g (mg/g)	2.4±0.9	2.4±1.3	2.2±1.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Quinic acid derivatives (mg/g)	10±1.5	8.5±3.2	9.5±1.9	0.6±0.5	0.6±0.5	0.5±0.5	0.5±0.3	0.6±0.3	0.6±0.3
Protein content (mg/g)	33.4±8.4	40.7±8.6	35.8±7.2	52.1±12.4	44.2±10.1	42.2±7.5	30.7±11.4	41.7±8.0	39.6±5.2