

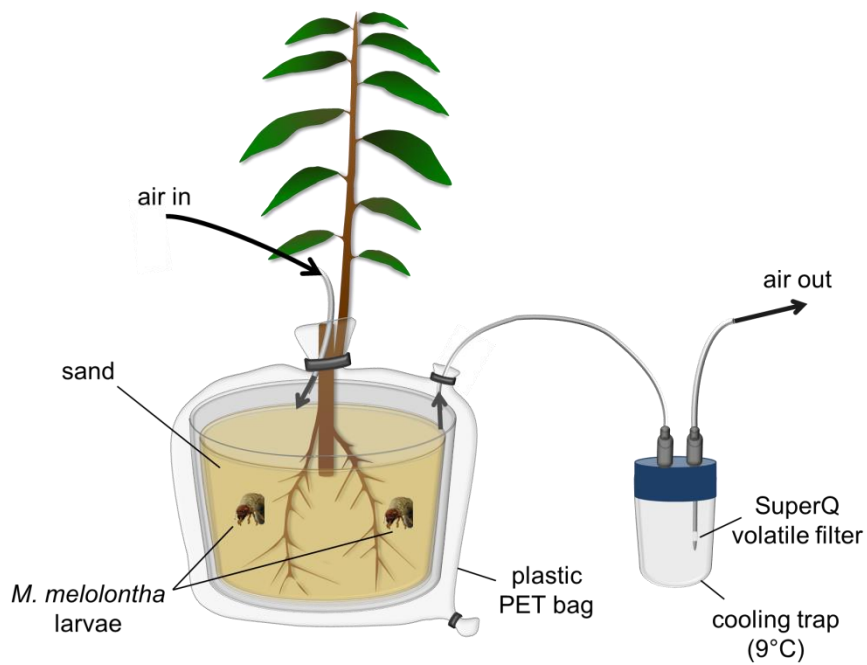
Supplementary material:

## **The occurrence and formation of monoterpenes in herbivore-damaged poplar roots**

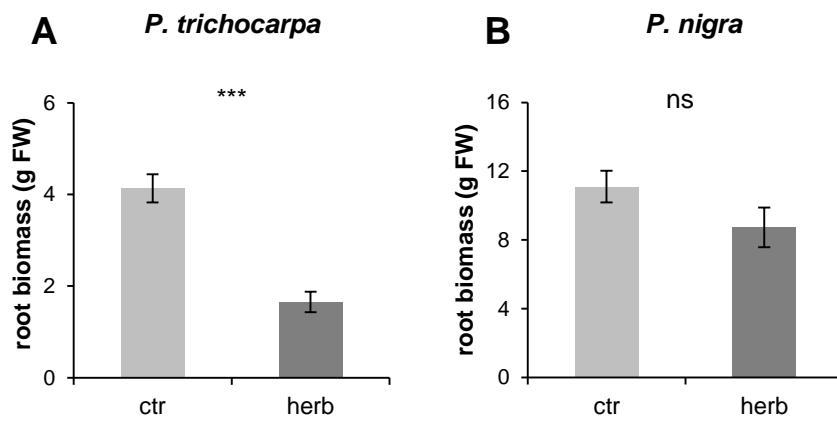
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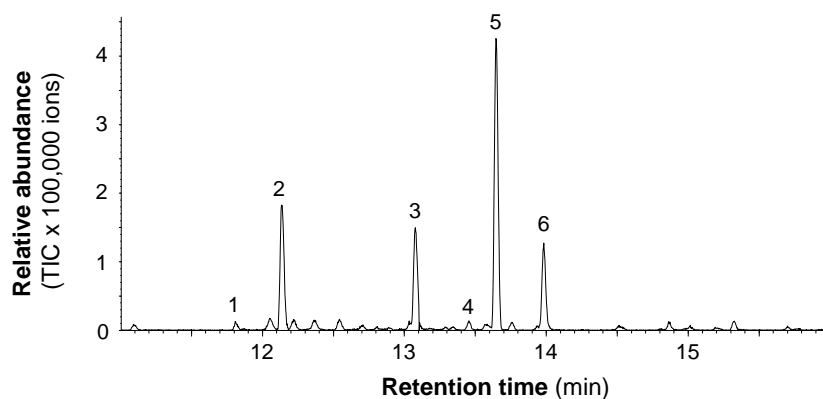


**Supplementary Figure S1: Root volatile collection system.** Volatiles were sampled for 68 h with a dynamic push-pull system and a trap packed with Poropak adsorbent. Trees were grown in sand and the pots were bagged in PET foil for volatile collection.



**Supplementary Figure S2: Effect of herbivory on root mass of *Populus trichocarpa* (A) and *P. nigra* (B) trees.** Root masses are displayed for undamaged roots (ctr) and roots damaged by cockchafer (*Melolontha melolontha*) larvae (herb) and shown as means  $\pm$  SE in g ( $n = 8$ ). Asterisks indicate statistical significance in Student's t-tests. *P. trichocarpa* ( $P \leq 0.001$ ,  $t = 6.973$ ); *P. nigra* ( $P = 0.108$ ,  $t = 1.717$ ).

## PnTPS4



**Supplementary Figure S3: Sesquiterpene synthase activity of PnTPS4.** The gene was heterologously expressed in *E. coli* and partially purified protein was incubated with (*E,E*)-FPP as substrate. Enzyme products were analyzed using GC-MS. 1, (*E*)- $\alpha$ -bergamotene; 2, (*E*)- $\beta$ -farnesene; 3, (*E,E*)- $\alpha$ -farnesene; 4, sesquiphellandrene; 5, (*Z*)- $\alpha$ -bisabolene; 6, nerolidol. Compounds were tentatively identified by database comparisons.

**A**

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### chlorop v1.1 prediction results #####
Number of query sequences: 3
```

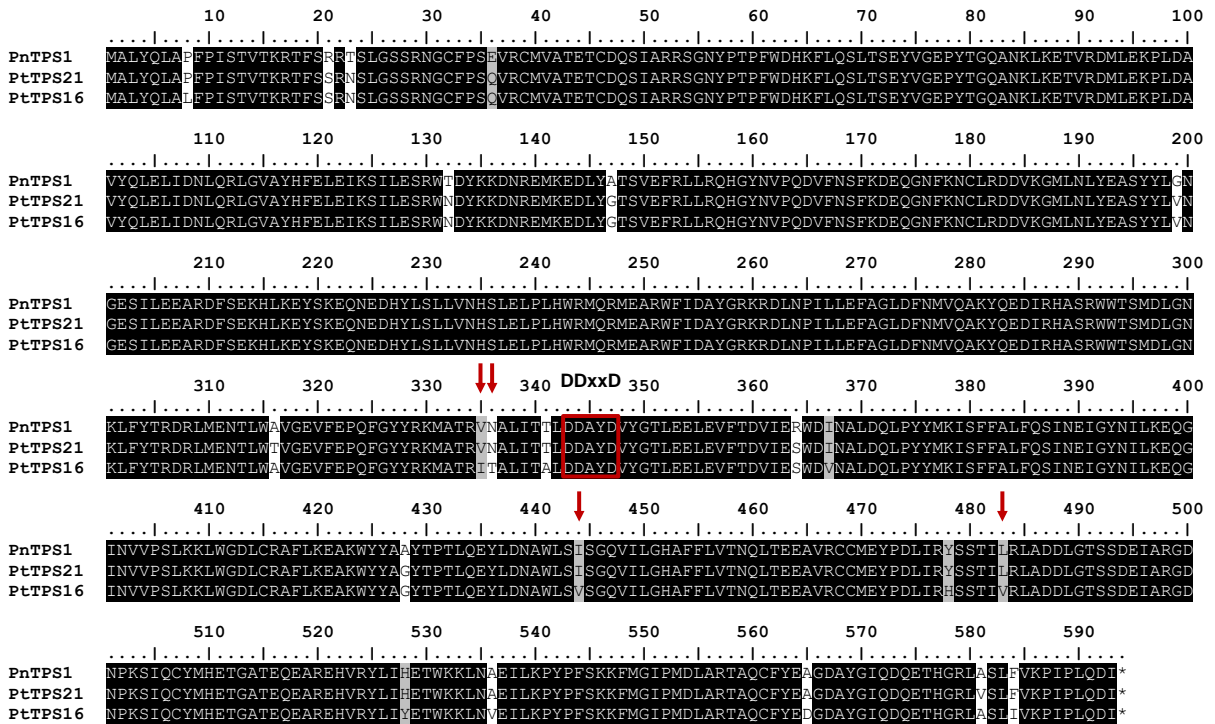
Name	Length	Score	cTP	CS-score	cTP-length
PnTPS4_full_length	589	0.568	Yes	11.435	40
PtTPS16_full_length	593	0.569	Yes	11.435	38
PtTPS21_full_length	593	0.570	Yes	11.435	38

**B**

```
### targetp v1.1 prediction results #####
Number of query sequences: 3
Cleavage site predictions not included.
Using PLANT networks.
```

Name	Len	cTP	mTP	SP	other	Loc	RC
PnTPS4_full_length	589	0.913	0.108	0.083	0.040	C	1
PtTPS16_full_length	593	0.826	0.486	0.029	0.016	C	4
PtTPS21_full_length	593	0.894	0.352	0.028	0.025	C	3
cutoff		0.000	0.000	0.000	0.000		

**Supplementary Figure S4: Signal peptide prediction of PnTPS4, PtTPS16, and PtTPS21.** Signal peptide (cTP) prediction was done using the web-based prediction programs chlorop v1.1 (<http://www.cbs.dtu.dk/services/ChloroP/>) (A) and targetp v1.1 (<http://www.cbs.dtu.dk/services/TargetP/>) (B).



**Supplementary Figure S5: Amino acid sequence comparison of PtTSP16 and PtTSP21 from *Populus trichocarpa* and PnTSP1 from *P. nigra*.** Amino acids with identical side chains are marked by black boxes and amino acids with similar side chains are marked by gray boxes. Red arrows highlight different amino acid residues in the active site. The conserved DDxxD motif is marked in red.

**Supplementary Table S1.** Emission of aromatic volatile compounds from undamaged (ctr) and *Melolontha melolontha*-damaged (herb) roots of *Populus trichocarpa* and *P. nigra*. Volatiles were analyzed using GC-MS/FID and emission levels are displayed as means  $\pm$  SE in  $\text{pg g}^{-1} \text{h}^{-1}$  fresh weight ( $n = 8$ ). *P*-values are based on the results from Student's t-tests or from Mann-Whitney Rank Sum Tests between control and herbivore treatments.

	<i>P. trichocarpa</i>				<i>P. nigra</i>			
	ctr	herb	<i>P</i> -value	t-value	ctr	herb	<i>P</i> -value	t-value/ <i>T</i> -value
benzaldehyde	123 $\pm$ 44	297 $\pm$ 98	0.063	-2.023	69 $\pm$ 15	75 $\pm$ 34	0.442	76.00
benzyl alcohol	244 $\pm$ 63	269 $\pm$ 61	0.765	-0.305	284 $\pm$ 68	364 $\pm$ 131	0.931	0.0887
salicylaldehyde	749 $\pm$ 401	1403 $\pm$ 631	0.236	-1.239	1467 $\pm$ 520	1002 $\pm$ 324	0.376	0.914

**Supplementary Table S2.** Accumulation of aromatic volatile compounds and camphene in undamaged (ctr) and *Melolontha melolontha*-damaged (herb) roots of *Populus trichocarpa* and *P. nigra*. Root material was extracted with hexane and analyzed using GC-MS/FID. Accumulation levels are displayed as means  $\pm$  SE in  $\mu\text{g g}^{-1}$  fresh weight (n = 8). *P*-values are based on the results from Student's t-tests or from Mann-Whitney Rank Sum Tests between control and herbivore treatments.

	<i>P. trichocarpa</i>				<i>P. nigra</i>			
	ctr	herb	<i>P</i> -value	t-value/ <i>T</i> -value	ctr	herb	<i>P</i> - value	t-value/ <i>T</i> -value
camphene	NA	NA	NA	NA	0.0 $\pm$ 0.0	0.3 $\pm$ 0.1	0.002	40.00
benzaldehyde	7.9 $\pm$ 4.4	48.9 $\pm$ 6.6	$\leq$ 0.001	37.00	0.1 $\pm$ 0.05	0.2 $\pm$ 0.03	0.373	-0.920
benzyl alcohol	11.9 $\pm$ 1.4	1.9 $\pm$ 1.9	$\leq$ 0.001	5.298	NA	NA	NA	NA
salicylaldehyde	550.1 $\pm$ 135.4	1948.6 $\pm$ 209.5	$\leq$ 0.001	-7.408	33.4 $\pm$ 4.3	73.4 $\pm$ 8.9	$\leq$ 0.001	-4.538



**Supplementary Table S3:** Oligonucleotides used for isolation, qRT-PCR analysis and site-directed mutagenesis of *TPS* genes.

<b>Name</b>	<b>Sequence</b>	<b>Usage</b>
PtTPS16-PtTPS21-fwd	CACCATGGTAGCGACCGAAACTTG	cloning of <i>PtTPS16/21</i>
PtTPS16-rev	CTATATATCTTGGAGAGGAATGG	cloning of <i>PtTPS16</i>
PtTPS21-rev	GATTTATCAAGAAAACCTTAAC	cloning of <i>PtTPS21</i>
PnTPS4-fwd	ATGGTAGGTCTCAGCGCGTTGCCACCGAAGCTGCTGGT	cloning of <i>PnTPS4</i>
PnTPS4-rev	ATGGTAGGTCTCATATCATAAAGGCTTAATAAGTAAGGATTC AC	cloning of <i>PnTPS4</i>
PnTPS1-PtTPS21-qRT-PCR- fwd	TATCGAAAAATGGCGACCAGGG	qRT-PCR
PnTPS1-PtTPS21-qRT-PCR-rev	GCAGTTGGTCCAATGCATTGAT	qRT-PCR
PtTPS16-qRT-PCR-fwd	TATCGAAAAATGGCGACCAGGA	qRT-PCR
PtTPS16-qRT-PCR-rev	GCAGTTGGTCCAATGCATTGAC	qRT-PCR
PnTPS4-qRT-PCR-fwd	CATATACAGCGCGAATCGAAAAGT	qRT-PCR
PnTPS4-qRT-PCR-rev	AGATAACCCAAGTCTTTGCAAGGC	qRT-PCR
PtTPS16-I335V-fwd	ATGGCGACCAGGGTTACTGCTCTAATAACAGCATTAGAT	site-directed mutagenesis
PtTPS16-I335V-rev	ATCTAATGCTGTTATTAGAGCAGTAACCCTGGTCGCCAT	site-directed mutagenesis
PtTPS16-T336N-fwd	ATGGCGACCAGGATTAATGCTCTAATAACAGCATTAGAT	site-directed mutagenesis
PtTPS16-T336N-rev	ATCTAATGCTGTTATTAGAGCATTAACTCTGGTCGCCAT	site-directed mutagenesis
PtTPS16-V444I-fwd	GATAATGCTTGGTTGTCAATTTCCGGACAAGTCATACTA	site-directed mutagenesis
PtTPS16-V444I-rev	TAGTATGACTTGTCGGAAATTGACAACCAAGCATTATC	site-directed mutagenesis
PtTPS16-V483L-fwd	CGTCACTCGTCAACGATTTTTCGACTCGCAGATGACCTA	site-directed mutagenesis
PtTPS16-V483L-rev	TAGGTCATCTGCGAGTCGAAAATCGTTGACGAGTGACG	site-directed mutagenesis
PtTPS16-I335V-T336N-fwd	ATGGCGACCAGGGTTAATGCTCTAATAACAGCATTAGAT	site-directed mutagenesis
PtTPS16-I335V-T336N-rev	ATCTAATGCTGTTATTAGAGCATTAAACCCTGGTCGCCAT	site-directed mutagenesis