

New Phytologist Supporting Information

Article title: Sniffing fungi – Phenotyping of volatile chemical diversity in *Trichoderma* species

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Table S3 Chemical identification and chromatographic characteristics of detected VOCs by Gas Chromatography Mass Spectrometry (GC-MS) analysis.

Table S4 Mean emission intensities of all the compounds detected by PTR-ToF-MS ($\text{ncps cm}^{-2} \text{s}^{-1}$) and GC-MS ($\text{pmol cm}^{-2} \text{h}^{-1}$) from *T. harzianum* (tha), *T. hamatum* (thm), *T. reesei* (tre) and *T. velutinum* (tve).

Fig. S1 Growth curves of the *Trichoderma* species. Grey arrows indicate the time point of PTR-ToF-MS measurement initiation. The time points were chosen to lie in the exponential growth stage of the fungi and were determined in preceding experiments from the maximum of the first derivative of the growth curve. The red dash line indicates the maximum growth area (i.e., a Petri dish filled by the mycelium); tha, *T. harzianum*; thm, *T. hamatum*; tre, *T. reesei*; tve, *T. velutinum*. Data is shown as means \pm se, n=6.

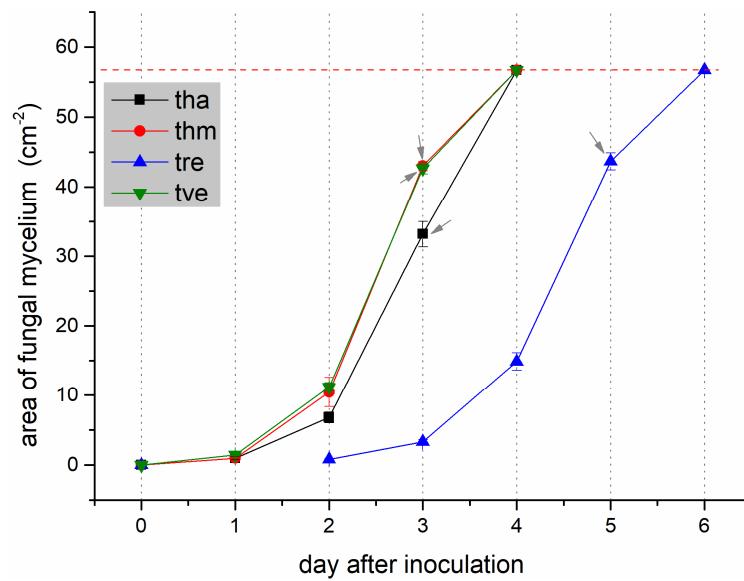


Fig. S2 Pictures of *Trichoderma* cultures at the onset and the end of the PTR-ToF-MS measurements.

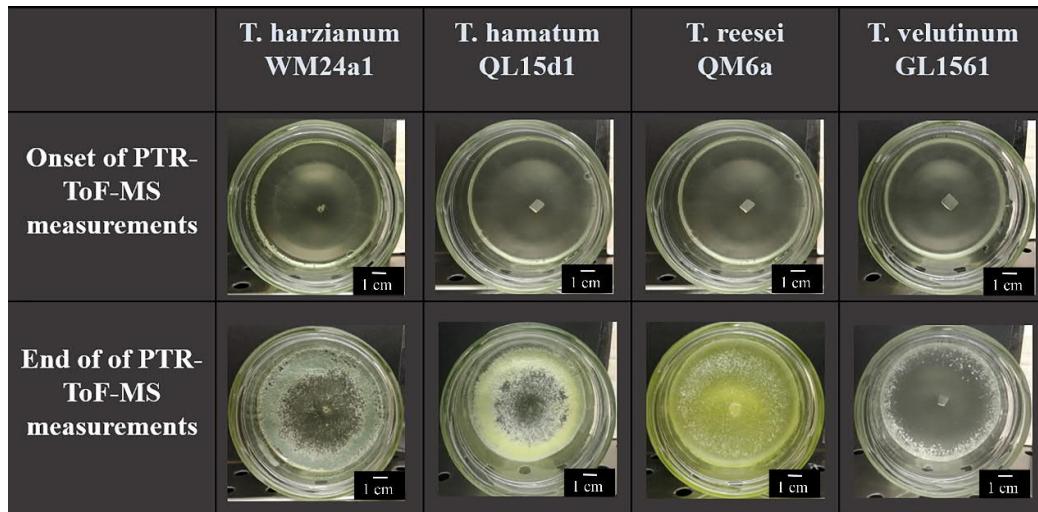


Fig. S3 Workflow. A fungal volatile organic compounds (fVOCs) screening experiment including measurements by PTR-ToF-MS and GC-MS instruments requires several working steps: fungi cultivation, fungal image photography, measurement of volatiles, raw data evaluation, background correction and further data mining. Prior to PTR-ToF-MS measurement, the entire fungal cuvettes system was sterilized with 80% of ethanol (prepared with analytical grade ethanol) and subsequently cleaned with sterile ultrapure water to avoid contamination.

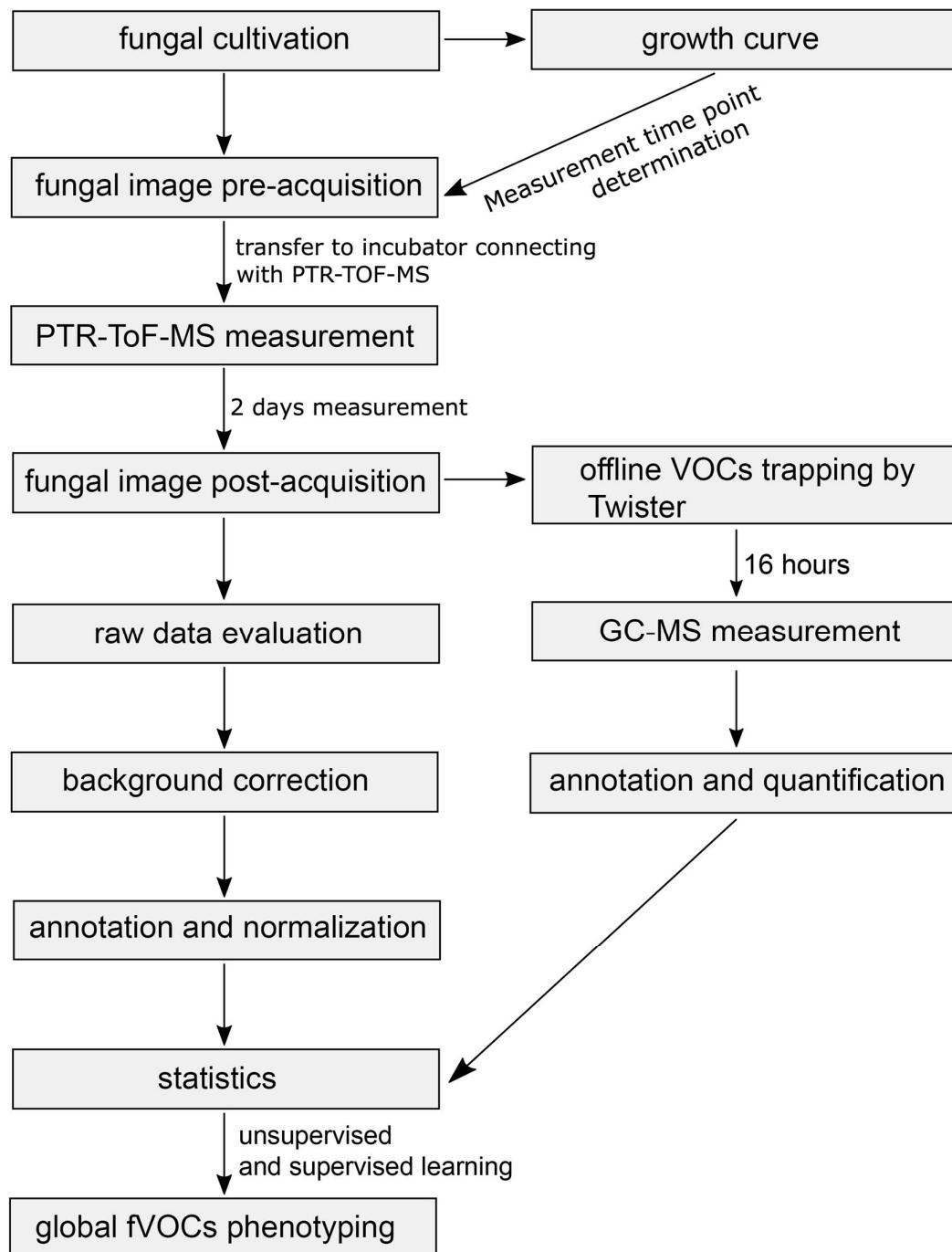


Fig. S4 Total emission intensity of sesquiterpenes detected by PTR-ToF-MS.

Sesquiterpenes (m/z 205.196) detected by PTR-ToF-MS from *T. harzianum* (tha), *T. hamatum* (thm), *T. reesei* (tre) and *T. velutinum* (tve). Data are means \pm se, $n=4$. Different letters on the bar plot indicate significant difference of their emission (one-way ANOVA Duncan's test, $p < 0.05$).

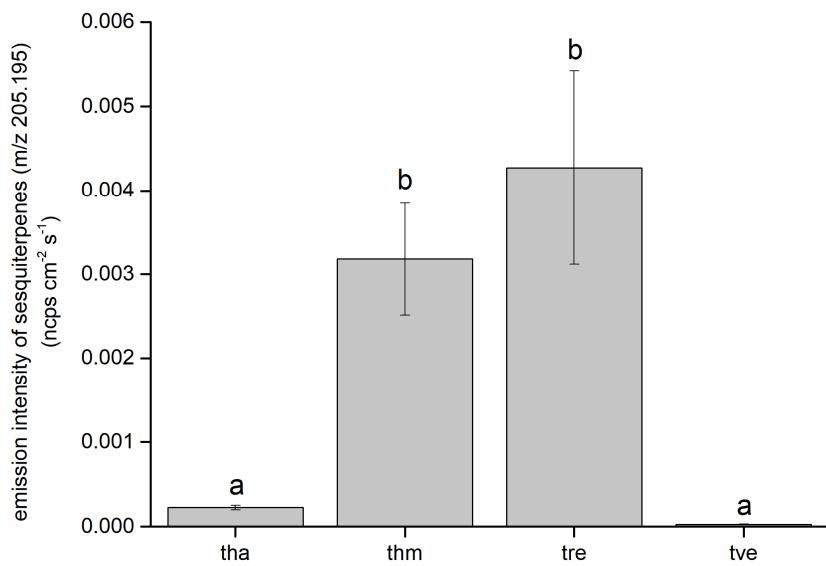


Fig. S5 Comparison of the volatile compounds emitted by the four *Trichoderma* species. Venn diagrams show volatiles detected by PTR-ToF-MS (a) and GC-MS (b) from *T. harzianum* (tha), *T. hamatum* (thm), *T. reesei* (tre) and *T. velutinum* (tve). Numbers in (b) refer to Supporting Information Table S3.

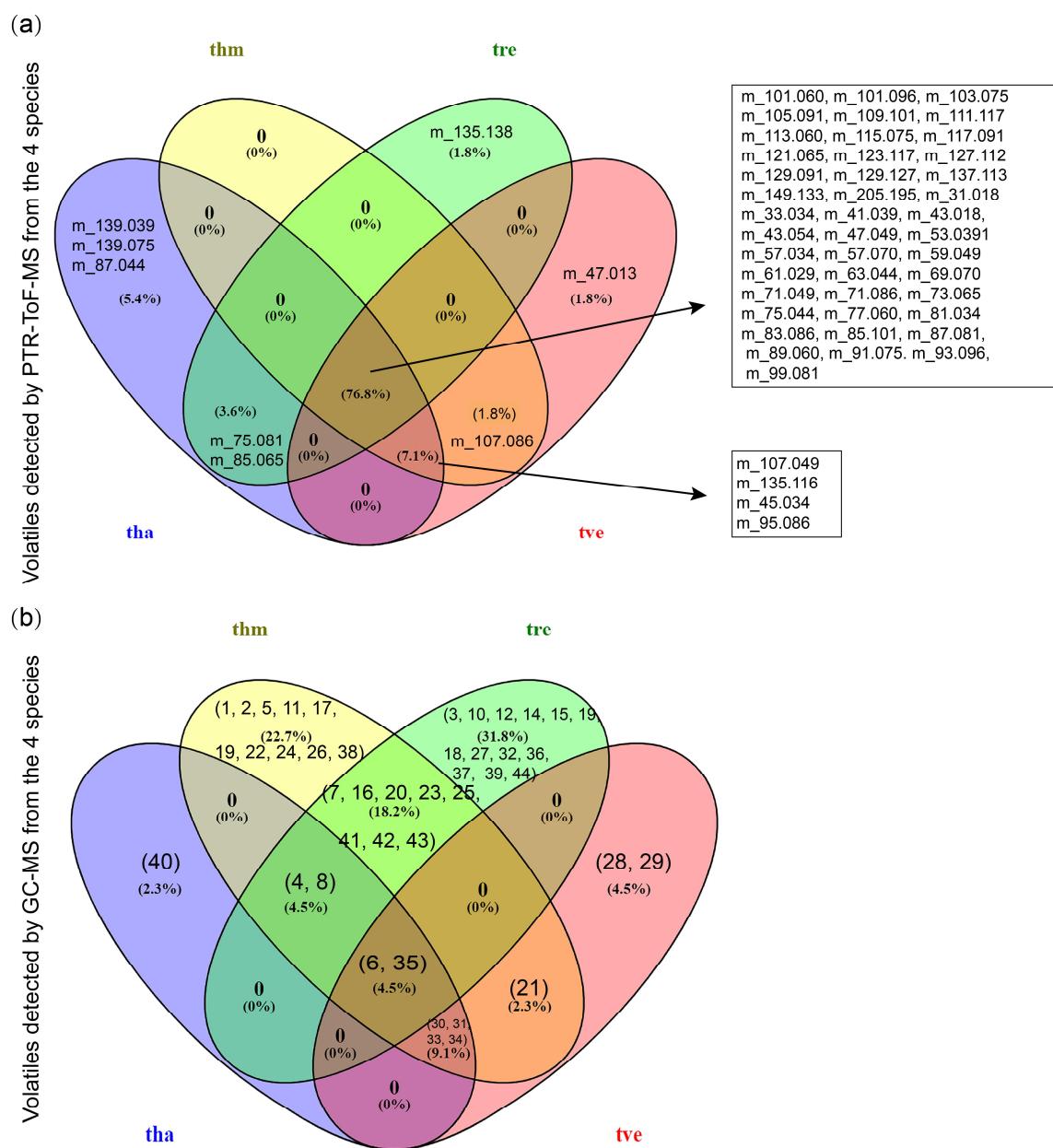


Table S1 The compounds (mass to charge ratios (m/z)) detected by Proton Transfer Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS) and corresponding tentative annotations. Isotopes and known common fragments were omitted. Same background colors in the “mass” column indicates the signals have strong correlations ($R^2 > 0.9$). The tentatively assigned compounds in red bold font are those detected previously in *Trichoderma* species and described in the literature. Footnotes refer to: ¹(Mancuso *et al.*, 2015); ²(Asensio *et al.*, 2007); ³(Infantino *et al.*, 2017); ⁴(Seewald *et al.*, 2010); ⁵(Misztal *et al.*, 2018); ⁶(Ladygina *et al.*, 2006); ⁷(Brilli *et al.*, 2011); ⁸(Bunge *et al.*, 2008); ⁹(Aprea *et al.*, 2015); ¹⁰(Mayrhofer *et al.*, 2006); ¹¹(Bäck *et al.*, 2010); ¹²(Lippolis *et al.*, 2014); ¹³(Morath *et al.*, 2012); ¹⁴(Maleknia *et al.*, 2007); ¹⁵(Demarcke *et al.*, 2009); ¹⁶(Kim *et al.*, 2009); ¹⁷(Minardi *et al.*, 2009). The references are studies that investigated soil and fungal volatile emission using PTR-ToF-MS.

mass (m/z)	sum formula	tentatively assigned compound	chemical class
31.018	$\text{CH}_2\text{O}+\text{H}^+$	formaldehyde ^{1, 2, 3}	aldehyde
33.034	$\text{CH}_4\text{O}+\text{H}^+$	methanol ^{1, 3, 4, 5}	alcohol
41.038	$\text{C}_3\text{H}_4+\text{H}^+$	alkyl fragment ³	n. i
43.018	$\text{C}_2\text{H}_2\text{O}+\text{H}^+$	acetic acid fragment³	acid
43.054	$\text{C}_3\text{H}_6+\text{H}^+$	alkyl fragment ^{3, 6}	n. i
45.034	$\text{C}_2\text{H}_4\text{O}+\text{H}^+$	acetaldehyde ^{1, 3, 7, 8}	aldehyde
47.013	$\text{CH}_2\text{O}_2+\text{H}^+$	formic acid ³	acid
47.049	$\text{C}_2\text{H}_6\text{O}+\text{H}^+$	ethanol^{1, 2, 3, 5}	alcohol
53.039	$\text{C}_4\text{H}_4+\text{H}^+$	alkyl fragment ³	n. i
57.034	$\text{C}_3\text{H}_4\text{O}+\text{H}^+$	prop-2-enal ³	aldehyde
57.070	$\text{C}_4\text{H}_8+\text{H}^+$	Fragment ⁹	n. i
59.049	$\text{C}_3\text{H}_6\text{O}+\text{H}^+$	acetone^{1, 3, 4}	ketone
61.029	$\text{C}_2\text{H}_4\text{O}_2+\text{H}^+$	acetic acid^{1, 8, 9}	acid
63.044	$\text{C}_2\text{H}_6\text{O}_2+\text{H}^+$	ethane-1, 2-diol	alcohol
69.070	$\text{C}_5\text{H}_8+\text{H}^+$	isoprene ^{1, 10}	acyclic alkene
71.049	$\text{C}_4\text{H}_6\text{O}+\text{H}^+$	butyric acid, dihydrofuran	acid
71.086	$\text{C}_5\text{H}_{10}+\text{H}^+$	cyclopentane/ pentene ⁹	al kane/acyclic alkene
73.065	$\text{C}_4\text{H}_8\text{O}+\text{H}^+$	butan-2-one/butyraldehyde^{5, 9}	ketone/aldehyde
75.044	$\text{C}_3\text{H}_6\text{O}_2+\text{H}^+$	propanoic acid ^{3, 9, 13}	acid
75.081	$\text{C}_4\text{H}_{10}\text{O}+\text{H}^+$	Butanol	alcohol
77.060	$\text{C}_3\text{H}_8\text{O}_2+\text{H}^+$	e. g. acetone-H3O⁺ cluster/ 2-methoxyethanol	ketone/alcohol

81. 034	C ₅ H ₈ O+H ⁺	e. g. fragment/ cycl openta- 2, 4-di en-1-one	n. i. / ketone
83. 086	C ₆ H ₁₀ +H ⁺	hexanal fragment/ 2, 3- di methyl -1, 3-butyadiene ⁹	al dehyde/ acycl ic al kene
85. 065	C ₅ H ₈ O+H ⁺	pentenal / pent-1-en-3-one/ cycl opentanone ^{1, 3}	al dehyde/ketone
85. 101	C ₆ H ₁₂ +H ⁺	hexanol fragment ⁹	al cohol
87. 044	C ₄ H ₈ O ₂ +H ⁺	butane-2, 3-di one/ butyrol actone ⁹	ketone
87. 081	C ₅ H ₁₀ O+H ⁺	pentanal / pentenol ^{9, 12}	al dehyde/al cohol
89. 060	C ₄ H ₈ O ₂ +H ⁺	butanol c aci d ^{9, 13}	aci d
91. 075	C ₄ H ₁₀ O ₂ +H ⁺	e. g. butanedi ol / 2- ethoxyethanol	al cohol
93. 091	C ₇ H ₈ +H ⁺	e. g. tol uene/ bi cycl o[3. 2. 0]hepta-2, 6- di ene	acycl ic al kene
95. 086	C ₇ H ₁₀ +H ⁺	2-norbornene/ bi cycl o[2. 2. 1]hept-2-ene ^{3, 14}	acycl ic al kene
99. 081	C ₆ H ₁₀ O+H ⁺	hexenal isomeres ⁹	al dehyde
101. 060	C ₅ H ₈ O ₂ +H ⁺	oxo-pentanal / pentane-2, 3- di one/ γ - val erol actone ^{3, 9}	al dehyde/ketone
101. 096	C ₆ H ₁₂ O+H ⁺	(3Z)-3-hexen-1-ol / hexanal	al cohol /al dehyde
103. 075	C ₅ H ₁₀ O ₂ +H ⁺	e. g. pentanoic aci d/ 3- methylbutanoic aci d/ valeric acid ^{3, 9}	aci d
105. 091	C ₅ H ₁₂ O ₂ +H ⁺	1, 5-pentanedi ol / 3- ethoxypropan-1-ol ⁹	al cohol
107. 049	C ₇ H ₆ O+H ⁺	benzal dehyde/ 2, 4, 6- cycl oheptatri en-1-one ^{3, 5, 9}	al dehyde/ketone
107. 086	C ₈ H ₁₀ +H ⁺	ethyl benzene/ xylene ^{3, 9}	benzenoi d
109. 065	C ₇ H ₈ O+H ⁺	benzy al cohol / methyl phenol / phenyl methanol ^{5, 9}	al cohol
109. 101	C ₈ H ₁₂ +H ⁺	4-ethenyl cycl ohexene ^{9, 14} , 2- octenal / octenol /1-octen- 3-one ⁹ fragment	acycl ic al kene
111. 117	C ₈ H ₁₄ +H ⁺	1-ethyl cycl ohexene ⁹ , octanal / 1-octen-3-ol / 3- octanone ⁹ fragment	acycl ic al kene
113. 060	C ₆ H ₈ O ₂ +H ⁺	cycl ohexanedi one isomere/ 2-ethyl -2H-furan-5-one	ketone
115. 070	C ₆ H ₁₀ O ₂ +H ⁺	hexanedi one isomer/ (Z)-4- hydroxyhex-3-en-2-one	ketone
117. 091	C ₆ H ₁₂ O ₂ +H ⁺	hexanol c aci d/ ethyl butanoate ⁹	aci d/ester
121. 065	C ₈ H ₈ O+H ⁺	2-phenyl acetal dehyde /acetophenone ⁵	al dehyde/ketone
123. 117	C ₉ H ₁₄ +H ⁺	2, 3- di methyl bi cycl o[2. 2. 1]hept- -2-ene ^{3, 15}	acycl ic al kene

127. 112	C ₈ H ₁₄ O+H ⁺	2-octenal / octenol / 1-octen-3-one⁹	aldehyde/ketone/alcohol
129. 091	C ₇ H ₁₂ O ₂ +H ⁺	2-buten-1-ol -3-methyl-acetate/ butyl acrylate	ester
129. 127	C ₈ H ₁₆ O+H ⁺	octanal / 1-octen-3-ol / 3-octanone⁹	aldehyde/ketone/alcohol
135. 116	C ₁₀ H ₁₄ +H ⁺	p-cymene/ o-cymene^{3, 9, 14}	benzenoid
135. 138	C ₇ H ₁₈ O ₂ +H ⁺	n. i	n. i
137. 113	C ₁₀ H ₁₆ +H ⁺	various monoterpenes⁹	monoterpene
139. 039	C ₇ H ₆ O ₃ +H ⁺	salicylic acid	acid
139. 075	C ₈ H ₁₀ O ₂ +H ⁺	2-phenoxyethanol /tyrosol ⁵	benzenoid/alcohol
149. 133	C ₁₁ H ₁₆ +H ⁺	pentyl benzene ^{3, 16}	benzenoid
205. 195	C ₁₅ H ₂₄ +H ⁺	various sesquiterpenes ^{3, 17}	sesquiterpene

Table S2 Limit of detection (LOD) of individual compounds related to potential emission rate that may be detected from fungi using the VOC platform (normalized to cm^{-2} mycelium area). The LOD was estimated based on standard error of estimate approach during the experimental background measurements and using 3σ of the signal intensities (PTR-ToF-MS) or of the peak-to-peak noise of the baseline around the analyte retention time (GC-MS). For PTR-ToF-MS, LOD indicates the average ($\pm \text{se}$) of multiple estimations during the whole experiment; the numbers (*) may be converted from ncps to ppbv ($\text{cm}^{-2} \text{ s}^{-1}$) knowing the sensitivity (ncps/ppbv) of the specific compounds (cf. Table S1). PTR-ToF-MS sensitivities calculated from calibration curves and obtained from measurements of VOC standards passing through the whole system and an empty cuvette were: methanol ($m/z = 33.034$): 7.3, acetaldehyde ($m/z = 45.034$): 40.5, ethanol ($m/z = 47.050$): 1.09, isoprene ($m/z = 69.0705$): 10.2, butan-2-one ($m/z = 73.065$): 30, benzene ($m/z = 79.055$): 14.5, toluene, ($m/z = 93.070$): 12.82, p-xylene ($m/z = 107.086$): 11.04, α -pinene ($m/z = 137.133$): 2.97.

mass detected by PTR-ToF-MS (m/z)	LOD* (ncps $\text{cm}^{-2} \text{ s}^{-1}$)	se* (ncps $\text{cm}^{-2} \text{ s}^{-1}$)	Compounds detected by GC-MS	LOD (pmol $\text{cm}^{-2} \text{ h}^{-1}$)
33.034	1.36E-06	4.36E-07	β -elemene	0.17E-02
41.039	5.11E-06	1.67E-06	unknown SQT#3	2.45E-05
31.018	4.42E-05	2.2E-05	phenol, 3-(1-methylethyl)-	0.75E-03
43.018	5.69E-06	1.46E-06	β -bisabolene	0.50E-03
43.055	1.69E-05	8.35E-06	selina-4(15),7(11)-diene	1.81E-06
45.034	1.49E-05	4.86E-06	zingiberene	6.72E-05
47.013	2.55E-06	4.12E-07	α -muurolene	0.17E-03
47.050	4.69E-06	1.06E-06	α -selinene	6.79E-06
53.039	2.41E-07	5.43E-08	3-octanene	0.46E-03
57.034	7.27E-07	9.98E-08	germacrene d	0.11E-02
57.070	7.92E-07	2.26E-07	β -curcumene	7.97E-05
61.029	3.86E-06	7.47E-07	trans- α -bisabolene	0.31E-03
63.045	2.73E-07	4.04E-08	dodecyl acrylate	0.57E-02
69.071	3.03E-07	5.77E-08	α -gurjunene	0.42E-03
71.086	2.56E-07	3.57E-08	β -caryophyllene	0.17E-03
73.065	1.47E-06	4.23E-07	epsilon-muurolene	9.71E-06
75.045	1.29E-05	1.09E-05	epizonarene	3.66E-05
75.081	3.65E-07	4.67E-08	butylhydroxytoluene	0.46E-03

77.060	1.91E-07	3.72E-08	α -cedrene	0.17E-03
81.034	7.85E-07	1.74E-07	α -copaene	6.52E-06
83.086	2.21E-07	4.1E-08	β -sesquiphellandrene	0.59E-03
85.065	2.18E-07	3.1E-08	trans- γ -bisabolene	3.67E-06
85.102	1.4E-07	2.42E-08	nerolidol	6.39E-06
87.045	4.9E-07	8.01E-08	unknown o-SQT#1	4.58E-06
87.081	3.79E-07	1.22E-07	unknown o-SQT#2	0.37E-03
89.060	6.25E-07	2.49E-07	unknown o-SQT#3	0.42E-03
91.076	2.32E-07	2.65E-08	unknown o-SQT#4	0.29E-03
93.092	3.74E-07	6.56E-08	Tetrahydrocarvone	0.21E-03
95.086	1.77E-07	1.88E-08	β -patchoulene	0.16E-03
99.081	2.14E-07	2.41E-08	dodecane	0.57E-03
101.060	2.61E-07	5E-08	α -himachalene	7.75E-06
101.097	1.82E-07	3.7E-08	β -himachalene	9.75E-07
103.076	2.04E-07	3.56E-08	α -amorphene	4.26E-06
105.092	1.11E-07	9.25E-09	2-isopropyl-5-methyl-9-methylene[4.4.0]dec-1-ene	3.80E-06
107.050	1.51E-07	2.46E-08	β -cedrene	8.48E-06
107.086	4.28E-07	1.31E-07	cyclohexanol,1-acetyl-2-ethylidene	0.24E-02
109.065	2.25E-07	5.47E-08	γ -muurolene	3.26E-05
109.102	1.77E-07	2.58E-08	palustrol	5.05E-06
111.117	1.38E-07	1.77E-08	isoledene	2.40E-06
113.060	2.84E-07	3.25E-08	spiro[4.5]dec-8-en-7-ol, 4,8-dimethyl-1-(1-methylethyl)-(3Z,5E,7E)-nonatetra-1,3,5,7-ene	0.1E-03
115.071	1.92E-07	3.01E-08		0.24E-03
117.092	1.33E-07	2.26E-08	γ -cadinene	4.97E-05
121.065	1.31E-07	2.28E-08	tridecane	0.22E-02
123.117	1.37E-07	2.05E-08	undecane	0.76E-02
127.112	1.9E-07	5.24E-08		
129.092	8.42E-08	8.06E-09		
129.128	1.75E-07	3.4E-08		
135.116	1.75E-07	3.37E-08		
135.139	9.83E-08	4.25E-08		
137.113	2.64E-07	3.44E-08		
139.040	1.36E-07	3.79E-08		
139.075	1.82E-07	5.98E-08		
149.133	1.65E-07	3.41E-08		
205.195	1.97E-07	3.48E-08		

Table S3 Chemical identification and chromatographic characteristics of detected VOCs by Gas Chromatography Mass Spectrometry (GC-MS) analysis. RT, Retention time; RI, Kovats retention index. Compounds in red font were reported from *Trichoderma* spp. using GC-MS in previous studies: ¹(Nieto-Jacobo *et al.*, 2017); ²(Guo *et al.*, 2019); ³(Lee *et al.*, 2016); ⁴(Müller *et al.*, 2013a); ⁵(Contreras-Cornejo *et al.*, 2014); ⁶(Kumar *et al.*, 2018); ⁷(Polizzi *et al.*, 2012); ⁸(Stoppacher *et al.*, 2010); ⁹(Srinivasa *et al.*, 2017); ¹⁰(Shahiri Tabarestani *et al.*, 2016); ¹¹(Hung *et al.*, 2013); ¹²(Siddiquee *et al.*, 2012).

Nr.	VOC	RT (min)	RI	Chemical class	CAS registry number
1	β -elemene ^{1,2}	19.434	1406.56	SQT	515-13-9
2	α -gurjunene ^{1,2}	19.915	1430.796	SQT	489-40-7
3	unknown SQT	20.025	1436.249	SQT	-
4	α -cedrene ^{2, 3}	20.104	1440.145	SQT	469-61-4
5	β -caryophyllene ¹	20.22	1445.836	SQT	87-44-5
6	β -curcumene ²	20.269	1447.351	SQT	451-56-9
7	α -copaene ²	20.379	1453.58	SQT	3856-25-5
8	β -cedrene ³	20.416	1455.372	SQT	546-28-1
9	α -amorphene ^{4, 5}	20.483	1458.61	SQT	483-75-0
10	β -patchoulene ⁶	20.812	1474.34	SQT	514-51-2
11	ϵ -muurolene	20.876	1477.369	SQT	30021-46-6
12	γ -muurolene ²	20.911	1479.021	SQT	30021-74-0
13	α -zingiberene ²	21.087	1487.284	SQT	495-60-3
14	α -himachalene	21.121	1488.871	SQT	3853-83-6
15	γ -cadinene ¹	21.221	1493.525	SQT	1460-97-5
16	α -muurolene ⁵	21.343	1499.17	SQT	10208-80-7
17	germacrene d ^{1, 2}	21.495	1506.019	SQT	23986-74-5
18	trans- α -bisabolene ⁷	21.568	1509.281	SQT	17627-44-0
19	α -selinene ²	21.745	1517.141	SQT	473-13-2
20	β -bisabolene ^{2, 3}	21.776	1518.511	SQT	495-61-4
21	selina-4(15), 7(11)-dienone ^{1, 2}	21.855	1521.991	SQT	103827-22-1
22	2-isopropyl-5-methyl-9-methyl-ene[4.4.0]dec-1-ene	21.951	1526.203	SQT	150320-52-8
23	β -sesquiphelandrene ^{2, 3, 8}	22.166	1535.563	SQT	20307-83-9
24	trans- γ -bisabolene	22.221	1537.941	SQT	495-62-5
25	epi-zonarene ^{9, 10}	22.318	1542.121	SQT	41702-63-0
26	isol edene ²	22.361	1543.968	SQT	95910-36-4
27	β -himachalene ^{2, 3, 11}	22.679	1557.507	SQT	1461-03-6
28	3-octanone ^{1, 2, 8}	10.887	983.9965	ketone	106-68-3
29	(3Z, 5E, 7E)-nonatetra-1, 3, 5, 7-ene	12.2	1049.361	al kene	81129-96-6
30	undecane ^{3, 12}	13.428	1103.045	al kane	1120-21-4

31	tetrahydrocarvone²	14. 237	1142. 657	ketone	59471-80
32	cycl ohexanol , 1-acetyl -2-ethyl i dene	14. 741	1166. 074	al kene	-
33	dodecane¹²	15. 532	1201. 414	al kane	112-40-3
34	tri decane¹²	17. 409	1301. 05	al kane	629-50-5
35	butyl hydroxytol uene	21. 635	1512. 264	benzoi d	128-37-0
36	phenol , 3-(1-methyl ethyl)-	22. 081	1531. 874	al cohol	618-45-1
37	nerol i dol	22. 904	1566. 964	SQT-al cohol	7212-44-4
38	pal ustrol¹	23. 691	1599. 275	SQT-al cohol	5986-49-2
39	spiro[4. 5]dec-8-en-7-ol , 4, 8-di methyl -1-(1-methyl ethyl)-	26. 131	1689. 562	SQT-al cohol	61050-89-3
40	dodecyl acrylate	26. 192	1691. 702	ester	2156-97-0
41	unknown-o-SQT-1	27. 912	1750. 825	SQT-al cohol	-
42	unknown-o-SQT-2	28. 345	1765. 135	SQT-al cohol	-
43	unknown-o-SQT-3	28. 439	1768. 211	SQT-al cohol	-
44	unknown-o-SQT-4	28. 869	1782. 146	SQT-al cohol	-

Table S4 Mean emission intensities of all the compounds detected by PTR-ToF-MS

(ncps cm⁻² s⁻¹) and GC-MS (pmol cm⁻² h⁻¹) from *T. harzianum* (tha), *T. hamatum* (thm), *T. reesei* (tre) and *T. velutinum* (tve). The numbers can be converted into ppbv cm⁻² s⁻¹ by dividing them with the PTR-ToF-MS' sensitivities towards the related compounds (cf. Table S1). Sensitivities of some selected, calibrated compounds are: methanol: 7.3 ncps/ppbv, acetaldehyde: 40.5 ncps/ppbv, acetone: 39.2 ncps/ppbv, isoprene: 10.2 ncps/ppbv, butan-2-one: 30 ncps/ppbv, benzene: 14.5 ncps/ppbv. Values are mean±se (n=4), n.d denotes not detected.

mass (m/z)	Emission intensities of compounds detected by PTR-ToF-MS (ncps cm ⁻² s ⁻¹)							
	tha	se	thm	se	tre	se	tve	se
31.018	3.69E-02	3.48E-03	6.46E-02	2.45E-03	8.57E-02	4.35E-03	5.85E-02	6.20E-03
33.034	5.15E-02	5.70E-03	1.83E-01	1.17E-02	6.19E-03	8.16E-04	1.12E-01	1.74E-02
41.039	4.46E-01	6.25E-02	1.81E-01	1.07E-02	7.92E-02	1.65E-02	2.58E-01	3.55E-02
43.018	6.32E-02	3.18E-03	1.68E-01	9.85E-03	1.24E-01	8.68E-03	1.91E-01	3.03E-02
43.054	4.01E-01	5.73E-02	1.36E-01	9.67E-03	5.91E-02	8.35E-03	2.04E-01	3.13E-02
45.034	9.59E-01	6.49E-02	1.97E+00	8.70E-02	n.d	n.d	6.41E-01	1.12E-01
47.013	n.d	n.d	n.d	n.d	n.d	n.d	9.49E-03	5.41E-03
47.049	1.32E+00	1.63E-01	2.36E+00	1.73E-01	4.62E+00	2.91E-01	1.62E+00	3.74E-01
53.039	4.60E-04	4.69E-05	3.42E-04	2.13E-05	5.19E-04	8.31E-05	2.93E-04	4.06E-05
57.034	1.01E-02	1.32E-03	6.06E-03	2.95E-04	2.37E-03	5.06E-04	8.16E-03	9.84E-04
57.07	1.39E-02	7.79E-04	3.01E-02	8.49E-04	7.96E-02	1.13E-02	2.39E-02	1.39E-03
59.049	3.26E+00	5.92E-01	9.40E+00	6.05E-01	3.61E+00	5.74E-01	1.08E+01	2.73E+00
61.029	2.64E-02	3.16E-03	9.20E-02	1.07E-02	7.53E-02	1.37E-02	1.24E-01	2.16E-02
63.044	7.00E-03	4.01E-04	1.31E-02	4.65E-04	1.03E-02	1.65E-04	5.29E-03	7.22E-04
69.07	6.53E-04	3.98E-05	1.26E-03	7.76E-05	2.31E-03	1.40E-04	8.74E-04	1.32E-04
71.049	4.60E-04	2.70E-05	1.60E-04	3.39E-05	8.41E-03	3.12E-03	1.09E-04	1.89E-05
71.086	4.70E-04	2.16E-05	1.13E-03	6.47E-05	7.62E-03	5.11E-04	5.42E-04	6.82E-05
73.065	7.36E-02	1.31E-02	6.27E-03	1.01E-03	2.03E-01	5.13E-02	4.85E-03	1.26E-03
75.044	3.95E-03	4.81E-04	1.77E-02	7.25E-04	3.85E-03	4.23E-04	2.15E-02	3.25E-03
75.081	4.91E-04	5.93E-05	n.d	n.d	9.11E-04	1.02E-04	n.d	n.d
77.06	4.05E-03	5.89E-04	9.43E-03	4.32E-04	4.65E-03	5.89E-04	1.04E-02	2.03E-03

81.034	2.10E-03	9.04E-05	1.67E-03	1.14E-04	6.41E-04	8.21E-05	8.67E-04	2.08E-04
83.086	8.31E-05	6.33E-06	4.17E-04	2.88E-05	1.95E-04	2.49E-05	2.64E-04	2.87E-05
85.065	n.d	n.d	2.68E-07	5.93E-08	n.d	n.d	1.86E-07	4.59E-08
85.101	6.76E-05	1.75E-06	4.74E-05	3.11E-06	1.29E-04	8.26E-06	1.07E-04	1.12E-05
87.044	6.31E-04	1.03E-04	n.d	n.d	n.d	n.d	n.d	n.d
87.081	1.06E-03	2.17E-04	3.44E-04	6.14E-05	2.16E-03	4.06E-04	4.90E-04	1.29E-04
89.06	2.08E-03	6.16E-05	2.84E-03	1.65E-04	3.97E-02	8.19E-03	2.82E-03	2.73E-04
91.075	2.18E-04	3.02E-05	1.71E-04	6.68E-06	6.76E-04	5.20E-05	1.26E-04	1.08E-05
93.096	1.06E-03	2.06E-04	2.50E-03	2.55E-04	5.91E-03	5.28E-04	1.20E-03	4.35E-04
95.086	6.17E-04	1.01E-04	2.14E-03	3.98E-04	n.d	n.d	1.53E-03	3.08E-04
99.081	1.79E-04	6.51E-06	1.81E-04	1.13E-05	1.21E-04	1.20E-05	1.04E-04	3.05E-06
101.06	1.13E-04	9.01E-06	1.80E-04	2.69E-05	3.64E-04	5.86E-05	1.11E-04	1.14E-05
101.096	6.36E-05	6.12E-06	1.04E-04	1.17E-05	1.11E-04	1.61E-05	5.92E-05	3.63E-06
103.075	1.83E-04	2.80E-06	1.68E-04	9.79E-06	1.10E-03	1.49E-04	1.14E-04	9.40E-06
105.091	5.15E-05	3.05E-06	1.10E-04	5.13E-06	4.93E-04	1.42E-05	9.66E-05	1.10E-05
107.049	6.60E-05	1.96E-06	4.21E-05	4.58E-06	n.d	n.d	2.00E-05	1.78E-06
107.086	3.63E-07	9.30E-08	1.58E-04	1.42E-05	3.05E-07	6.38E-08	6.05E-05	1.02E-05
109.101	2.32E-04	2.21E-05	1.41E-03	2.56E-04	2.52E-03	6.59E-04	7.86E-05	6.19E-06
111.117	3.30E-05	5.93E-06	8.29E-05	1.09E-05	2.24E-04	4.78E-05	5.83E-05	1.53E-05
113.06	9.84E-05	6.11E-06	8.99E-05	5.52E-06	1.85E-04	2.30E-05	5.94E-05	2.11E-06
115.075	6.80E-05	1.34E-06	1.03E-04	1.09E-05	8.39E-05	1.42E-05	3.60E-05	4.35E-06
117.091	7.25E-05	2.54E-06	2.01E-04	1.92E-05	1.13E-04	8.79E-06	7.89E-05	1.04E-05
121.065	7.57E-05	2.56E-06	1.22E-04	4.60E-06	1.58E-04	2.79E-05	5.97E-05	6.31E-06
123.117	3.62E-05	2.24E-06	4.08E-04	6.35E-05	2.90E-04	6.01E-05	1.33E-05	1.23E-06
127.112	5.33E-05	2.87E-06	1.06E-04	3.42E-06	9.03E-05	1.53E-05	1.07E-04	1.76E-05
129.091	4.94E-05	7.79E-06	2.75E-05	1.67E-06	8.50E-05	1.39E-05	2.10E-05	3.84E-06
129.127	2.16E-04	5.35E-05	2.92E-04	2.25E-05	2.64E-04	6.00E-05	4.71E-04	8.33E-05
135.116	5.94E-05	3.89E-06	4.14E-04	6.90E-05	n.d	n.d	1.46E-05	1.04E-06
135.138	n.d	n.d	n.d	n.d	5.58E-04	1.28E-04	n.d	n.d
137.113	4.23E-05	2.29E-06	2.74E-04	5.46E-05	2.10E-04	2.53E-05	2.58E-05	2.81E-06
139.039	1.81E-05	1.58E-06	n.d	n.d	n.d	n.d	n.d	n.d
139.075	2.65E-05	1.05E-06	n.d	n.d	n.d	n.d	n.d	n.d
149.133	6.36E-05	6.38E-06	9.25E-04	1.72E-04	7.19E-04	1.80E-04	9.17E-06	7.29E-08
205.195	2.25E-04	2.83E-05	3.18E-03	6.68E-04	4.27E-03	1.15E-03	2.89E-05	1.69E-06

compound	Emission intensities of compounds detected by GC-MS (pmol cm ⁻² h ⁻¹)							
	tha	se	thm	se	tre	se	tve	se
(3Z,5Z,7E)-nona-1,3,5,7-tetraene	n.d	n.d	n.d	n.d	n.d	n.d	1.92E+00	3.33E-01
2-isopropyl-5-methyl-9-methylene[4.4.0]dec-1-ene	n.d	n.d	5.36E-01	1.90E-01	n.d	n.d	n.d	n.d
3-octanone	n.d	n.d	n.d	n.d	n.d	n.d	1.65E+00	2.37E-01
a-amorphene	n.d	n.d	n.d	n.d	9.78E-01	2.40E-02	n.d	n.d
a-cedrene	4.88E-02	1.73E-02	2.24E-01	8.25E-03	1.70E+00	2.38E-01	n.d	n.d
a-copaene	n.d	n.d	3.04E-01	5.81E-02	8.56E-01	3.64E-02	n.d	n.d
a-gurjunene	n.d	n.d	1.28E-01	3.19E-02	n.d	n.d	n.d	n.d
a-himachalene	n.d	n.d	n.d	n.d	1.96E-01	1.11E-02	n.d	n.d
a-muurolene	n.d	n.d	2.07E-01	1.25E-02	8.10E-01	2.47E-02	n.d	n.d
a-selinene	n.d	n.d	3.91E+00	2.82E-01	n.d	n.d	n.d	n.d
b-bisabolene	n.d	n.d	2.26E+00	1.41E-01	5.27E-01	5.00E-02	n.d	n.d
b-caryophyllene	n.d	n.d	1.11E+00	2.24E-01	n.d	n.d	n.d	n.d
b-cedrene	2.48E-02	7.69E-03	4.43E-01	7.27E-02	1.56E+00	6.59E-02	n.d	n.d
b-curcumene	7.13E-02	2.28E-02	2.81E-01	9.74E-03	2.00E+00	3.09E-01	8.17E-03	2.53E-03
b-elemene	n.d	n.d	5.21E+00	5.82E-01	n.d	n.d	n.d	n.d
b-himachalene	n.d	n.d	n.d	n.d	4.43E-02	4.06E-03	n.d	n.d
b-patchoulene	n.d	n.d	n.d	n.d	7.64E-02	2.54E-03	n.d	n.d
b-sesquiphellandrene	n.d	n.d	8.28E-01	1.56E-01	1.55E-01	1.19E-02	n.d	n.d
butylhydroxytoluene	1.88E+00	8.23E-01	5.90E+00	1.00E+00	2.76E+00	4.94E-01	8.86E+00	3.68E+00
cyclohexanol,1-acetyl-2-ethylidene	n.d	n.d	n.d	n.d	7.20E+00	2.47E-01	n.d	n.d
dodecane	1.86E-01	2.82E-02	1.67E-01	1.87E-02	n.d	n.d	1.71E-01	7.51E-02
dodecyl acrylate	2.68E-01	4.42E-02	n.d	n.d	n.d	n.d	n.d	n.d
epizonarene	n.d	n.d	2.66E-01	5.63E-02	1.01E-01	2.98E-03	n.d	n.d
epsilon -muurolene	n.d	n.d	1.52E-01	3.03E-02	n.d	n.d	n.d	n.d
gamma-cadinene	n.d	n.d	n.d	n.d	1.01E-01	5.64E-03	n.d	n.d
gamma-muurolene	n.d	n.d	n.d	n.d	1.03E-01	2.29E-03	n.d	n.d
germacrene d	n.d	n.d	8.78E-01	1.73E-01	n.d	n.d	n.d	n.d
isoledene	n.d	n.d	3.30E-01	6.77E-02	n.d	n.d	n.d	n.d
nerolidol	n.d	n.d	n.d	n.d	2.61E+00	6.81E-02	n.d	n.d
palustrol	n.d	n.d	9.96E-02	1.64E-02	n.d	n.d	n.d	n.d
phenol, 3-(1-methylethyl)-	n.d	n.d	n.d	n.d	1.10E+01	4.81E-01	n.d	n.d
selina-4(15),7(11)-diene	n.d	n.d	1.05E+00	1.64E-01	n.d	n.d	9.26E-03	2.85E-03
spiro[4.5]dec-8-en-7-ol, 4,8-dimethyl-1-(1-methylethyl)-tetrahydrocarvone	n.d	n.d	n.d	n.d	2.30E+00	2.23E-01	n.d	n.d
	2.67E+00	4.00E-01	5.24E+00	1.62E+00	n.d	n.d	1.59E-01	8.59E-03

trans-a-bisabolene	n.d	n.d	n.d	n.d	3.23E-01	1.41E-02	n.d	n.d
trans-gamma-bisabolene	n.d	n.d	1.01E+01	2.08E+00	n.d	n.d	n.d	n.d
tridecane	1.96E-01	2.52E-02	1.75E-01	1.29E-02	n.d	n.d	1.93E-01	7.23E-02
undecane	1.05E+00	2.70E-01	7.04E-01	9.68E-02	n.d	n.d	1.19E+00	4.01E-01
unknown SQT	n.d	n.d	n.d	n.d	9.05E-01	5.07E-02	n.d	n.d
unknown_o_SQT_1	n.d	n.d	1.24E-02	2.92E-03	5.40E-01	9.76E-02	n.d	n.d
unknown_o_SQT_2	n.d	n.d	1.59E-02	3.77E-03	1.46E-01	1.91E-02	n.d	n.d
unknown_o_SQT_3	n.d	n.d	3.95E-02	1.08E-02	5.65E-01	8.01E-02	n.d	n.d
unknown_o_SQT_4	n.d	n.d	n.d	n.d	8.26E-02	2.91E-02	n.d	n.d
zingiberene	n.d	n.d	n.d	n.d	5.57E-01	1.26E-02	n.d	n.d

References

- Aprea E, Romano A, Betta E, Biasioli F, Cappellin L, Fanti M, Gasperi F.** 2015. Volatile compound changes during shelf life of dried *Boletus edulis*: comparison between SPME - GC - MS and PTR - ToF - MS analysis. *Journal of Mass Spectrometry* **50**: 56-64.
- Asensio D, Peñuelas J, Filella I, Llusia J.** 2007. On-line screening of soil VOCs exchange responses to moisture, temperature and root presence. *Plant and Soil* **291**: 249-261.
- Bäck J, Aaltonen H, Hellén H, Kajos MK, Patokoski J, Taipale R, Pumpanen J, Heinonsalo J.** 2010. Variable emissions of microbial volatile organic compounds (MVOCs) from root-associated fungi isolated from Scots pine. *Atmospheric Environment* **44**: 3651-3659.
- Brilli F, Ruuskanen TM, Schnitzhofer R, Müller M, Breitenlechner M, Bittner V, Wohlfahrt G, Loreto F, Hansel A.** 2011. Detection of plant volatiles after leaf wounding and darkening by proton transfer reaction “time-of-flight” mass spectrometry (PTR-TOF). *PLoS One* **6**: e20419.
- Bunge M, Araghipour N, Mikoviny T, Dunkl J, Schnitzhofer R, Hansel A, Schinner F, Wisthaler A, Margesin R, Märk TD.** 2008. On-line monitoring of microbial volatile metabolites by proton transfer reaction-mass spectrometry. *Applied and Environmental Microbiology* **74**: 2179-2186.
- Contreras-Cornejo HA, Macías-Rodríguez L, Herrera-Estrella A, López-Bucio J.** 2014. The 4-phosphopantetheinyl transferase of *Trichoderma virens* plays a role in plant protection against *Botrytis cinerea* through volatile organic compound emission. *Plant and Soil* **379**: 261-274.
- Demarcke M, Amelynck C, Schoon N, Dhooghe F, Van Langenhove H, Dewulf J.** 2009. Laboratory studies in support of the detection of sesquiterpenes by proton-transfer-reaction-mass-spectrometry. *International Journal of Mass Spectrometry* **279**: 156-162.
- Guo Y, Ghirardo A, Weber B, Schnitzler J-P, Benz JP, Rosenkranz M.** 2019. *Trichoderma* species differ in their volatile profiles and in antagonism toward ectomycorrhiza *Laccaria bicolor*. *Frontiers in Microbiology* **10**: 891.
- Hung R, Lee S, Bennett JW.** 2013. Arabidopsis thaliana as a model system for testing the effect of *Trichoderma* volatile organic compounds. *Fungal Ecology* **6**: 19-26.

- Infantino A, Costa C, Aragona M, Reverberi M, Taiti C, Mancuso S. 2017.** Identification of different *fusarium* spp. through mVOCs profiling by means of proton-transfer-reaction time-offlight (PTR-TOF-MS) analysis. *Journal of Plant Pathology* **99**: 663-669.
- Kim S, Karl T, Helming D, Daly R, Rasmussen R, Guenther A. 2009.** Measurement of atmospheric sesquiterpenes by proton transfer reaction-mass spectrometry (PTR-MS). *Atmospheric Measurement Techniques* **2**: 99-112.
- Kumar SM, Chowdappa P, Gore R. 2018.** Volatile organic compounds emitted by *Trichoderma harzianum* OTPB3 elicited antifungal activity against *Phytophthora infestans* and induces plant growth promotion and systemic resistance in tomato. *International Journal of Innovative Horticulture* **7**: 128-138.
- Ladygina N, Dedyukhina E, Vainshtein M. 2006.** A review on microbial synthesis of hydrocarbons. *Process Biochemistry* **41**: 1001-1014.
- Lee S, Yap M, Behringer G, Hung R, Bennett JW. 2016.** Volatile organic compounds emitted by *Trichoderma* species mediate plant growth. *Fungal Biology and Biotechnology* **3**: 7.
- Lippolis V, Pascale M, Cervellieri S, Damascelli A, Visconti A. 2014.** Screening of deoxynivalenol contamination in durum wheat by MOS-based electronic nose and identification of the relevant pattern of volatile compounds. *Food Control* **37**: 263-271.
- Maleknia SD, Bell TL, Adams MA. 2007.** PTR-MS analysis of reference and plant-emitted volatile organic compounds. *International Journal of Mass Spectrometry* **262**: 203-210.
- Mancuso S, Taiti C, Bazihizina N, Costa C, Menesatti P, Giagnoni L, Arenella M, Nannipieri P, Renella G. 2015.** Soil volatile analysis by proton transfer reaction-time of flight mass spectrometry (PTR-TOF-MS). *Applied Soil Ecology* **86**: 182-191.
- Mayrhofer S, Mikoviny T, Waldhuber S, Wagner AO, Innerebner G, Franke - Whittle IH, Märk TD, Hansel A, Insam H. 2006.** Microbial community related to volatile organic compound (VOC) emission in household biowaste. *Environmental Microbiology* **8**: 1960-1974.
- Minerdi D, Bossi S, Gullino ML, Garibaldi A. 2009.** Volatile organic compounds: a potential direct long - distance mechanism for antagonistic action of *Fusarium oxysporum* strain MSA 35. *Environmental Microbiology* **11**: 844-854.

- Misztal PK, Lymeropoulou DS, Adams RI, Scott RA, Lindow SE, Bruns T, Taylor JW, Uehling J, Bonito G, Vilgalys R.** 2018. Emission factors of microbial volatile organic compounds from environmental bacteria and fungi. *Environmental Science & Technology* **52**: 8272-8282.
- Morath SU, Hung R, Bennett JW.** 2012. Fungal volatile organic compounds: a review with emphasis on their biotechnological potential. *Fungal Biology Reviews* **26**: 73-83.
- Müller A, Faubert P, Hagen M, Zu Castell W, Polle A, Schnitzler JP, Rosenkranz M.** 2013a. Volatile profiles of fungi--chemotyping of species and ecological functions. *Fungal Genetics and Biology* **54**: 25-33.
- Nieto-Jacobo MF, Steyaert JM, Salazar-Badillo FB, Nguyen DV, Rostás M, Braithwaite M, De Souza JT, Jimenez-Bremont JF, Ohkura M, Stewart A.** 2017. Environmental growth conditions of *Trichoderma* spp. affects indole acetic acid derivatives, volatile organic compounds, and plant growth promotion. *Frontiers in Plant Science* **8**: 102.
- Polizzi V, Adams A, De Saeger S, Van Peteghem C, Moretti A, De Kimpe N.** 2012. Influence of various growth parameters on fungal growth and volatile metabolite production by indoor molds. *Science of the Total Environment* **414**: 277-286.
- Seewald MS, Singer W, Knapp BA, Franke-Whittle IH, Hansel A, Insam H.** 2010. Substrate-induced volatile organic compound emissions from compost-amended soils. *Biology and Fertility of Soils* **46**: 371-382.
- Shahiri Tabarestani M, Rahnama K, Jahanshahi M, Nasrollahnejad S, Fatemi M.** 2016. Identification of volatile organic compounds of some *Trichoderma* species using static headspace gas chromatography-mass spectrometry. *Mycologia Iranica* **3**: 47-55.
- Siddiquee S, Cheong BE, Taslima K, Kausar H, Hasan MM.** 2012. Separation and identification of volatile compounds from liquid cultures of *Trichoderma harzianum* by GC-MS using three different capillary columns. *Journal of Chromatographic Science* **50**: 358-367.
- Srinivasa N, Sriram S, Singh C, Shivashankar K.** 2017. Secondary metabolites approach to study the bio-efficacy of *Trichoderma asperellum* isolates in india. *International Journal of Current Microbiology and Applied Sciences* **6**: 1105-1123.
- Stoppacher N, Kluger B, Zeilinger S, Krska R, Schuhmacher R.** 2010. Identification and profiling of volatile metabolites of the biocontrol fungus *Trichoderma atroviride* by HS-SPME-GC-MS. *Journal of Microbiological Methods* **81**: 187-193.