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Supporting Information

for

Volatile emission and biosynthesis in endophytic fungi colonizing black poplar leaves

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Sequences of isolated endophytic fungi and identification according to NCBI database, primer used in this study, representative total ion chromatograms of single endophytic volatile blend, mass spectra of unknown volatile organic compounds, and BUSCO analysis of *Cladosporium* sp. de novo assembly

Contents

Table S1: Endophytes were identified to genus level via sequencing of ribosomal DNA (ITS1F/ ITS4). The obtained sequences were compared to the NCBI sequence database and the identity (%) of best hits with their accession number is given in the main document.

Species	Sequence
Alternaria infectoria	TGTCTTTTGCGTACTTCTTGTTTCCTGGGTGGGCTCGCCCGCCCTCAGGACCAAC CACAAACCTTTTGCAATAGCAATCAGCGTCAGTAACAACGTAATTAATTACAACTT TCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA CGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG CCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT TTGCTTGGTGTTGGGCGTCTTTTGTCTCCAGTTCGCTGGAGACTCGCCTTAAAGT CATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCGCTCTTCGCC AGCCAAGGTCAGCGTCCAGCAAGCCTTTTTTTCAACCTTTGACCTCGGATCAGGT AGGGATACCCG
Alternaria sp. 1	TTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAA TTGCAATCAGTGTCAGTAACAAATTAATAATTACAACTTTCAACAACGGATCTCTT GGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCA GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAA GGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGC GTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTA CTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATC CATTAAGCCTTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAAC TTAAGCATATCAATAAGCGGAGGA
Stemphylium sp.	AAAAATGTGGTCTTGATGGATGCTCAACCAAGGCCGATTCAAAGTGCAAGAATTG TGCTGCGCTCCGAAACCAGTAGGTCGGCTGCCAATCATTTTAAGGCGAGTCTCG TGAGAGACAAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTC GAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGCGTTCAAAGATTCG ATGATTCACTGAATTCTGCAATTCACACTACGTATCGCATTTCGCTGCGTTCTTCA TCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATAATTACATTGTTTAC TGACGCTGATTGCAATTACAAAAAGGTTTATGGTTTGGTCCTGGTGGCGGGCGAA CCCGCCCAGGAAACAAGAAGTGCGCAAAAGACATGGGTGAATAATTCAGACAAG CTGGAGCCCTCACCGAGGTGAGGTCCCAACCCGCTTTCATATTGTGTAATGATCC CTCCGCAGGTTCACC
Aureobasidium sp. 1	GTCCCAGGCGAGCGCCCGCCAGAGTTAAACCAAACTCTTGTTATTTAACCGGTC GTCTGAGTTAAAATTTTGAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTC GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAG TGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGAGGGGCATG CCTGTTCGAGCGTCATTACACCACTCAAGCTATGCTTGGTATTGGGCGTCGTCCT TAGTTGGGCGCGCCTTAAAGACCTCGGCGAGGCCACTCCGGCTTTAGGCGTAGT AGAATTTATTCGAACGTCTGTCAAAGGAGAGGAACTCTGCCGACTGAAACCTTTA TTTTTCTAGGTTGACCTCGGATCAGGTAGGGATACCC

Species	Sequence
Aureobasidium sp. 2	ATAAAGGTTTCAGTCGGCAGAGTTCCTCTCCTTTGACAGACGTTCGAATAAATTCT ACTACGCCTAAAGCCGGAGTGGCCTCGCCGAGGTCTTTAAGGCGCGCCCAACTA AGGACGACGCCCAATACCAAGCATAGCTTGAGTGGTGTAATGACGCTCGAACAG GCATGCCCCTCGGAATACCAAGGGGCGCAATGTGCGTTCAAAGATTCGATGATT CACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATG CGAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTTATTCAAAATTTTAACTCAG ACGACCGGTTAAATAACAAGAGTTTGGTTTAACTCTGGCGGGCGCTCGCCTGGG ACGAATCCCCAGCGGCTCGAGACCGAGCGGTCCCGCCAAAGCAACAAGGTAGTT TTAACAACAAAGGGTTGGAGGTCGGGCGCTGAGCACCCTTACTCTTTAATGATCC TTCCGCAGGTTCACCTACGGAAGNGGATNATTAAAGAGTAAGGGTGCTCAGCGC CCGACCTCCAACCCTTTGTTGTTAAAACTACCTTGTTGCTTTGGCGGGACCGCTC GGTCTCGAGCCGCTGGGATTCGTCCCAGGCGAGCGCCCGCCAGAGTTAAACCA AACTCTTGTTATTTAACCGGTCGTCTGAGTTAAAATTTNGAATAAATNAAAACTTTN ACAACGGANCTCTTGGTTCTCGCATCGA
Didymella glomerata	CCGCCGATTGGNCAATTTAAACNATTTGCAGTTGCAATCAGCGTCTGAAAAAACT TAATAGTTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC AGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT GAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCAT TTGTACCTTCAAGCTCTGCTTGGTGTTGGGTGTTTGTCTCGCCTCTGCGTGTAGA CTCGCCTCAAAACAATTGGCAGCCGGCGTATTGATTTCGGAGCGCAGTACATCTC GCGCTTTGCACTCATAACGACGACGTCCAAAAGTACATTTTTACACTCTTGACCTC GGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGG
Didymella sp. 1	CCGCCGATTGGACAATTTAAACCATTTGCAGTTGCAATCAGCGTCTGAAAAAACTT AATAGTTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC AGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT GAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCAT TTGTACCTTCAAGCTCTGCTTGGTGTTGGGTGTTTGTCTCGCCTCTGCGTGTAGA CTCGCCTCAAAACAATTGGCAGCCGGCGTATTGATTTCGGAGCGCAGTACATCTC G
Didymella sp. 2	CTTTTAAGTACCTTACGTTTCCTCGGCGGGTCCGCCCGCCGATTGGACAATTTAA ACCATTTGCAGTTGCAATCAGCGTCTGAAAAAACTTAATAGTTACAACTTTCAACA ACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGT GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTT GGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCTTCAAGCTCTGCTT GGTGTTGGGTGTTTGTCTCGCCTCTGCGTGTAGACTCGCCTCAAAACAATTGGCA GCCGGCGTATTGATTTCGGAGCGCAGTACATCTCGCGCTTTGCACTCATAACGAC GACGTCCAAAAGTACATT
Cladosporium sp.	TCGGGCGGGGGCTCCGGGTGGACACTTCAAACTCTTGCGTAACTTTGCAGTCTG AGTAAACTTAATTAATAAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATC GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT CATCGAATCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTG TTCGAGCGTCATTTCACCACTCAAGCCTCGCTTGGTATTGGGCAACGCG
Fusarium sp.	GGGACGGCCCGCCGCAGGAAACCCTAAACTCTGTTTTTAGTGGAACTTCTGAGT ATAAAAAACAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGA TGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCA TCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTC GAGCGTCATTTCAACCCTCAAGCCCAGCTTGGTGTTGGGATCTGTGTGCAAACAC AGTCCCCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTA
Sordaria sp.	CGGGCCCCCGGATCCTCGGGTCTCCCGCTCGCGGGAGGCTGCCCGCCGGAGT GCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAAACTTTTAAATAAGTCA AAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA TTGCGCTCGCCAGTATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCATC AAGCTCTGCTTGCGTTGGGGATCCGCGTCTGACGCGGTCCCTCAAAAACAGTGG CGGGCTCGCTAGTCACACCGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGG CGGGTTCTTGCCGTAAAACCCCCAATTTCTAAGGTTGACCTCGGATCAGGTAGGA ATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

Species	Sequence
Arthrinium sp.	AAAAATGTGGTCTTGATGGATGCTCAACCAAGGCCGATTCAAAGTGCAAGAATTG TGCTGCGCTCCGAAACCAGTAGGTCGGCTGCCAATCATTTTAAGGCGAGTCTCG TGAGAGACAAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTC GAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGCGTTCAAAGATTCG ATGATTCACTGAATTCTGCAATTCACACTACGTATCGCATTTCGCTGCGTTCTTCA TCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATAATTACATTGTTTAC TGACGCTGATTGCAATTACAAAAAGGTTTATGGTTTGGTCCTGGTGGCGGGCGAA CCCGCCCAGGAAACAAGAAGTGCGCAAAAGACATGGGTGAATAATTCAGACAAG CTGGAGCCCTCACCGAGGTGAGGTCCCAACCCGCTTTCATATTGTGTAATGATCC CTCCGCAGGTTCACC

Table S2: Primers used in this study.

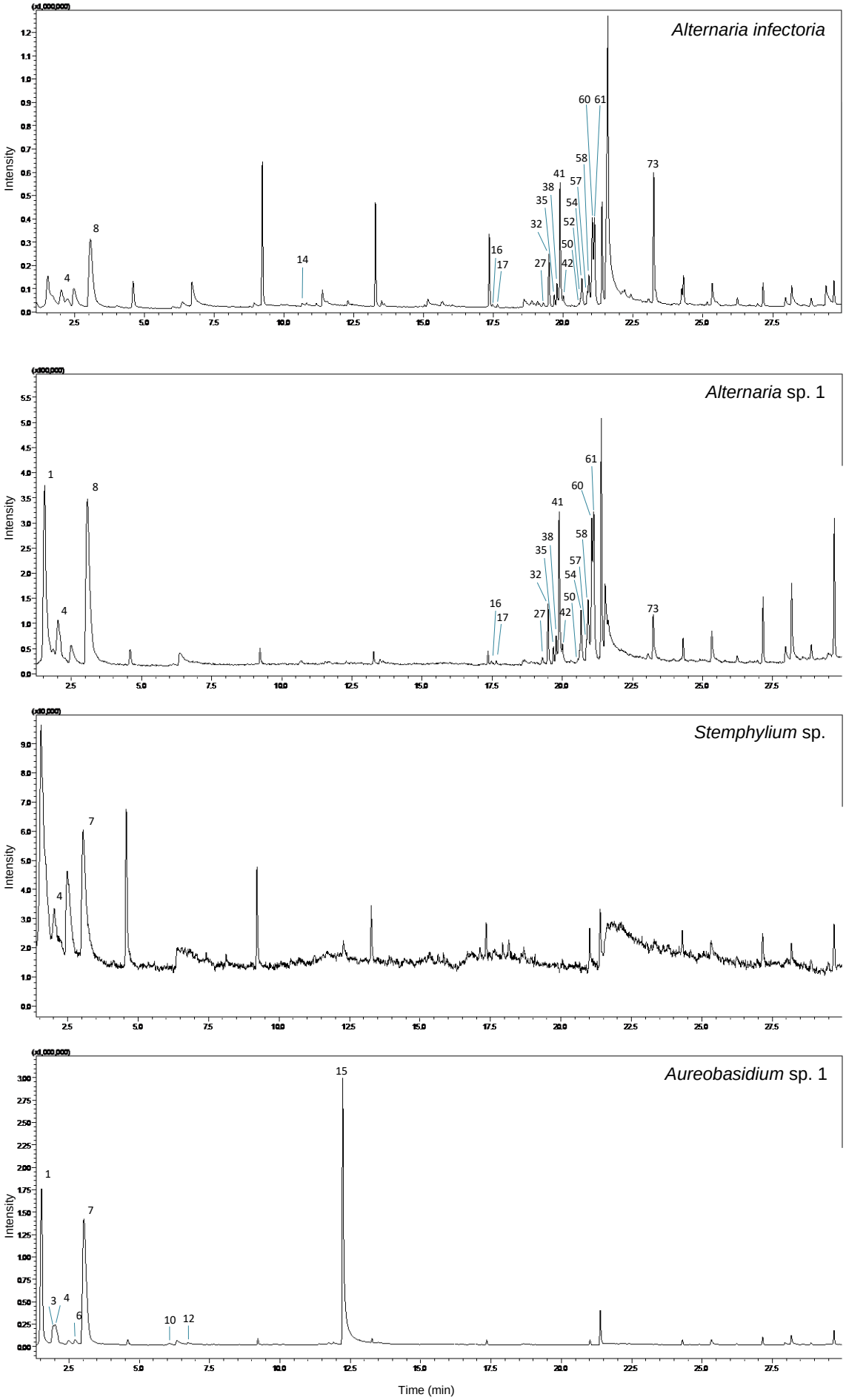
Name	Sequence	Purpose
CxTPS1_Fwd	CACCATGAGCTCTAGCACGGGTC	cloning
CxTPS1_Rev	TCACGACGCCCCTCG	cloning
CxTPS2_Fwd	CACCATGTCAGACCCTACTCGCC	cloning
CxTPS2_Rev	TCAGCAACACTCCAGATAGCTAGG	cloning
ITS1F	CTTGGTCATTTAGAGGAAGTAA	amplify fungal rRNA ITS
ITS4	TCCTCCGCTTATTGATATGC	amplify fungal rRNA ITS

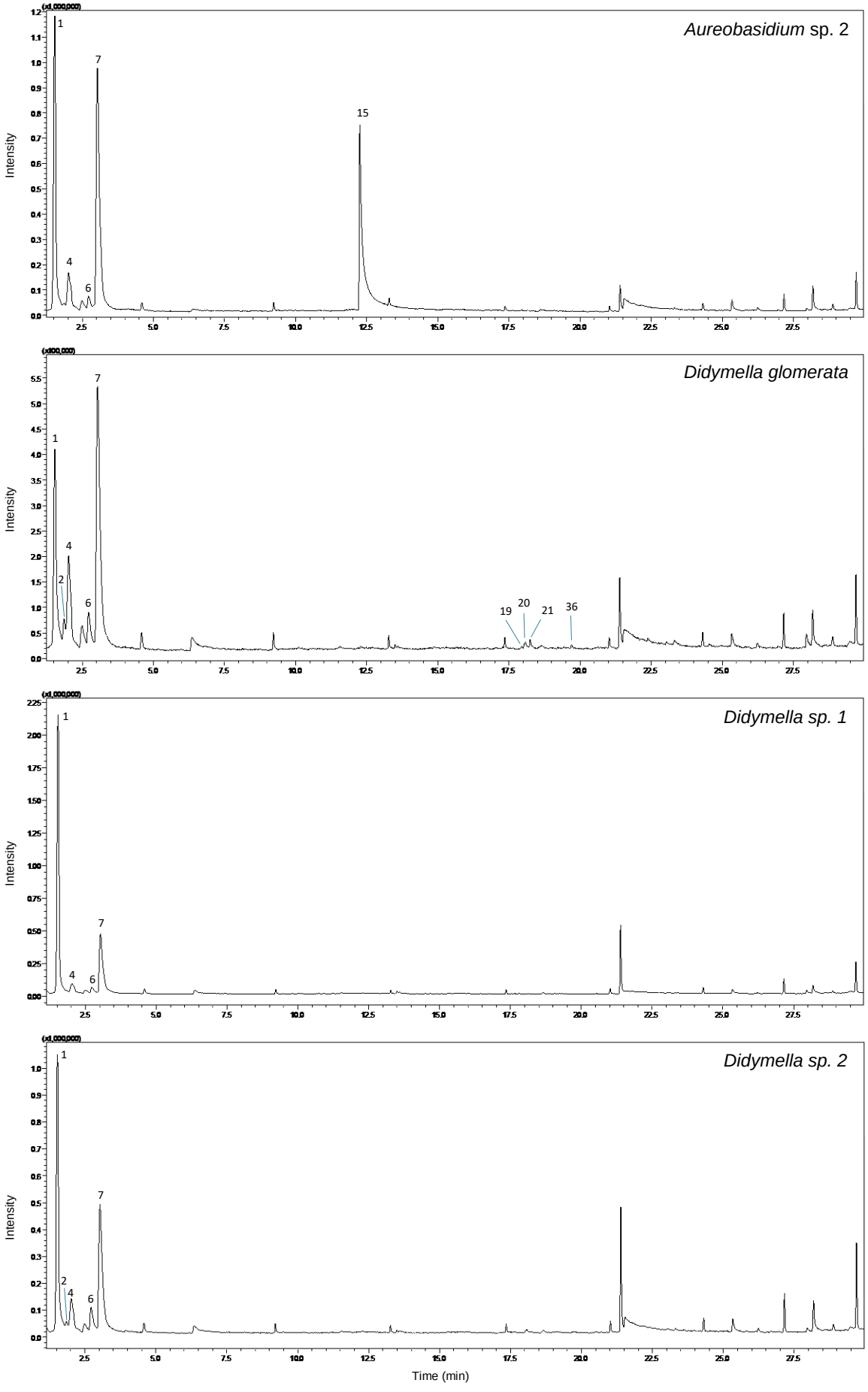
Table S3: Numbers for volatile organic compounds, which are shown in the total ion chromatograms of the volatile bouquet for each endophytic fungus in Figure S1.

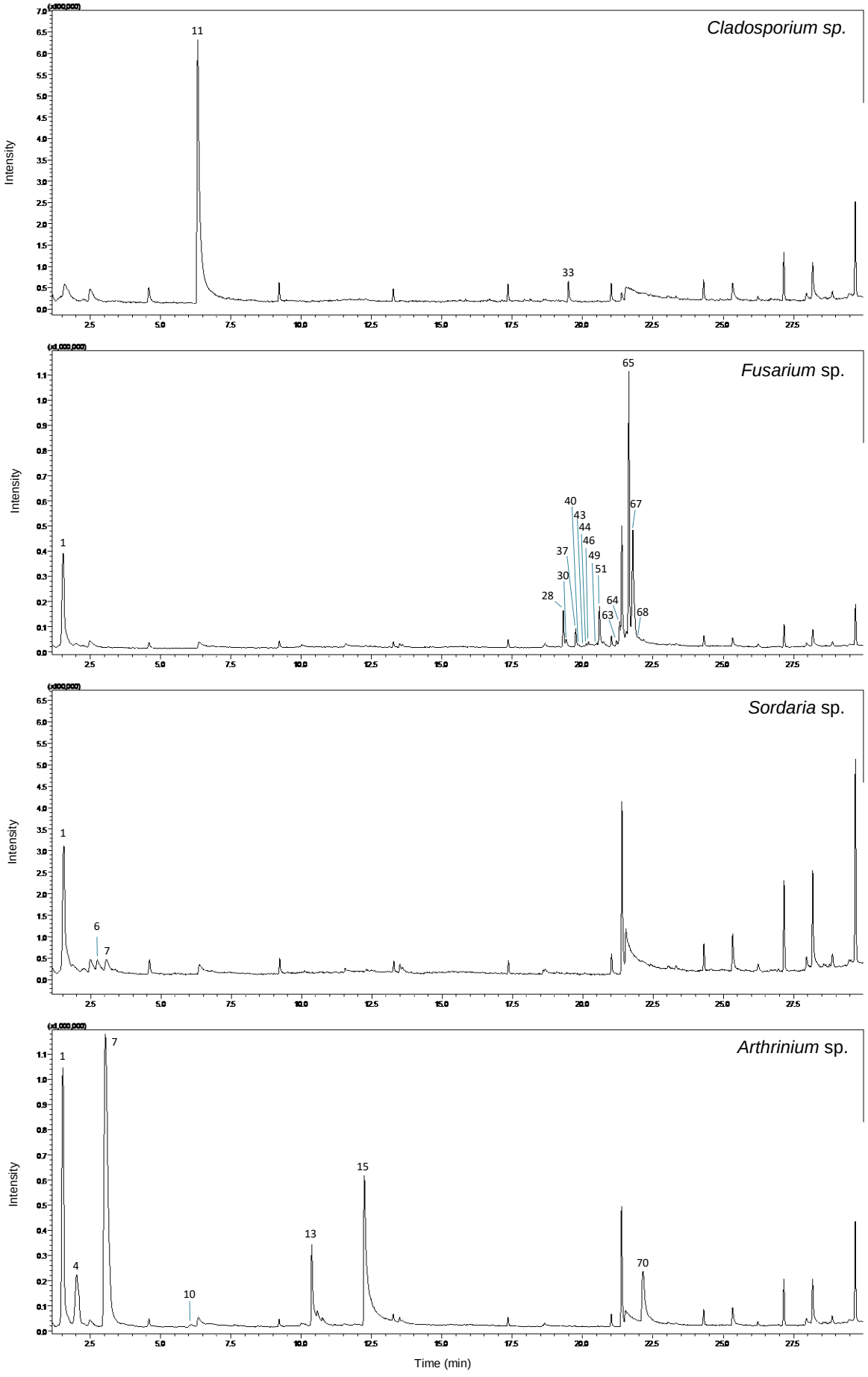
#	Volatile organic compound	R.T. (min)
1	Ethanol	1.525
2	2-Butanone	1.855
3	Ethyl Acetate	1.940
4	2-Methyl-1-propanol	2.020
5	unknown 1	2.265
6	3-Hydroxy-2-Butanone	2.720
7	3-Methyl-1-butanol	3.035
8	2-Methyl-1-butanol	3.070
9	unknown 2	3.780
10	3-Methylbutyl acetate	6.100
11	Ethenylbenzene	6.325
12	unknown 3	6.730
13	unknown 4	10.380
14	unknown 5	10.655
15	2-Phenylethanol	12.240
16	unknown 6	17.465
17	unknown 7	17.650
18	α-Cubebene	17.920

19	unknown 8	17.950
20	unknown 9	18.075
21	unknown 10	18.245
22	unknown 11	18.330
23	α-Copaene	18.525
24	unknown 12	18.765
25	unknown 13	18.850
26	Sativene	18.985
27	α-Gurjunene	19.280
28	unknown 14	19.300
29	unknown 15	19.380
30	unknown 16	19.400
31	unknown 17	19.460
32	Aristolene	19.485
33	(*E*)-β-Caryophyllene	19.500
34	unknown 18	19.535
35	unknown 19	19.675
36	unknown 20	19.695
37	Bicyclosesquiphellandrene	19.745
38	β-Gurjunene	19.775
39	unknown 21	19.790
40	unknown 22	19.845
41	unknown 23	19.880
42	α-Guaiene	20.000
43	unknown 24	20.015
44	unknown 25	20.120
45	unknown 26	20.140
46	(*E*)-β-Farnesene	20.195
47	unknown 27	20.325
48	unknown 28	20.440
49	unknown 29	20.490
50	unknown 30	20.555
51	β-Chamigrene	20.585
52	unknown 31	20.610
53	unknown 32	20.610
54	α-Selinene	20.665
55	γ-Muurolene	20.685
56	unknown 33	20.800
57	unknown 34	20.860
58	β-Selinene	20.910
59	unknown 35	20.930
60	(+)-Valencene	21.040
61	unknown 36	21.115
62	α-Muurolene	21.170
63	β-Himachalene	21.195
64	β-Bisabolene	21.305
65	unknown 37	21.630

66	unknown 38	21.635
67	unknown 39	21.775
68	unknown 40	21.985
69	unknown 41	22.075
70	unknown 42	22.150
71	unknown 43	22.355
72	unknown 44	22.720
73	unknown 45	23.235
74	unknown 46	23.875
75	unknown 47	24.485
76	unknown 48	24.675
77	unknown 49	25.980


Alternaria infectoria
Alternaria sp. 1
Stemphylium sp.
Aureobasidium sp. 1
Intensity
Time (min)


Aureobasidium sp. 2
Didymella glomerata
Didymella sp. 1
Didymella sp. 2
Intensity
Time (min)


Cladosporium sp.
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Intensity
Fusarium sp.
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40
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44
46
37
49
51
30
28
64
63
67
68
1
Sordaria sp.
1
6
7
Arthrinium sp.
1
7
15
13
4
10
70
Time (min)

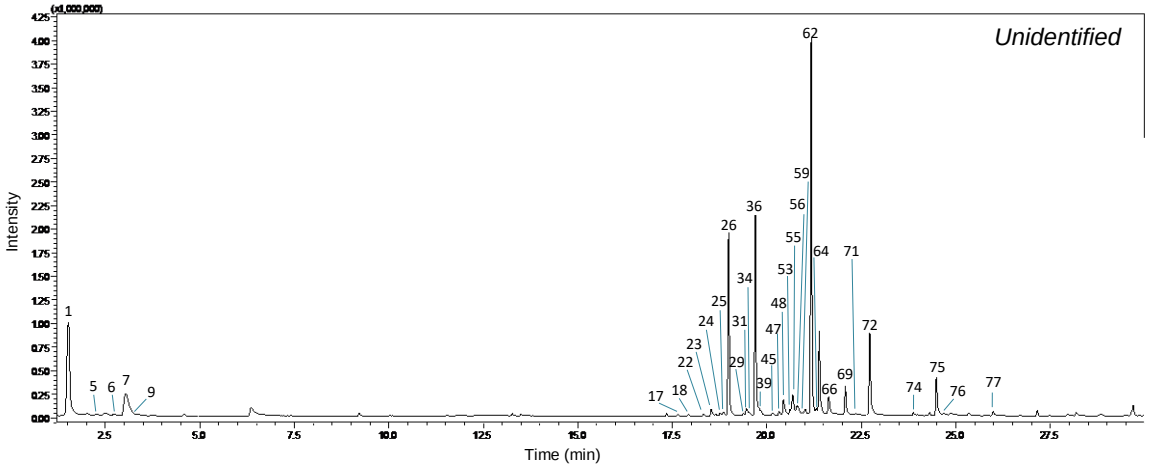
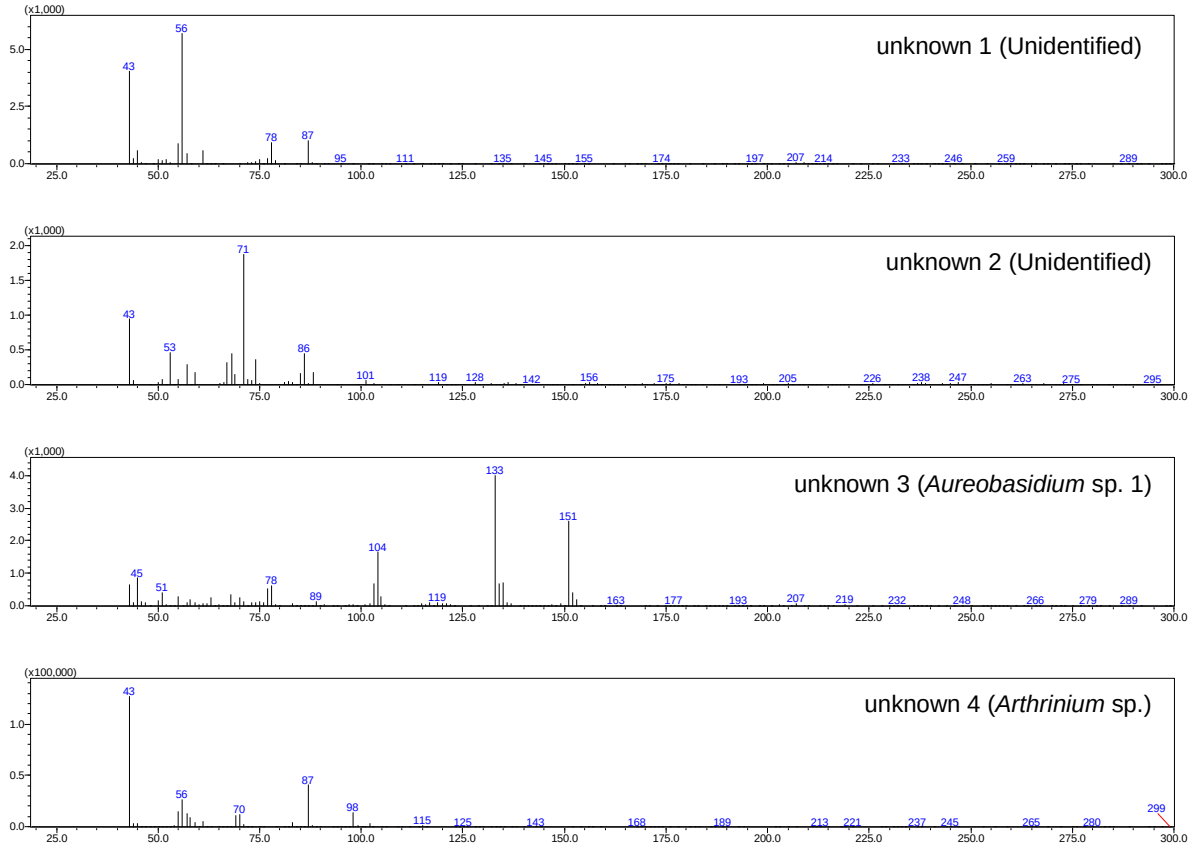
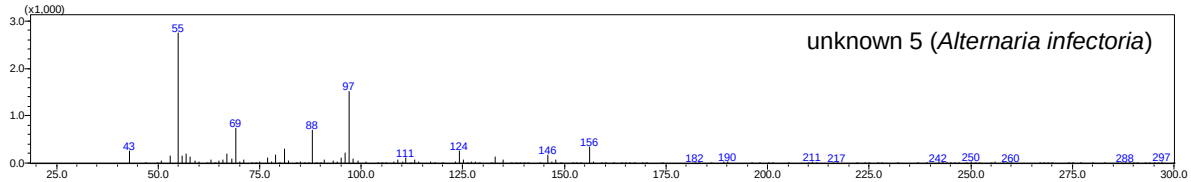


Figure S1: Representative total ion chromatogram of volatiles measured from different endophytes used in this study. Numbers indicate different volatile organic compounds, listed in Table S3. Peaks without numbers are either contamination from the PDMS tube or originate from the culture media itself.

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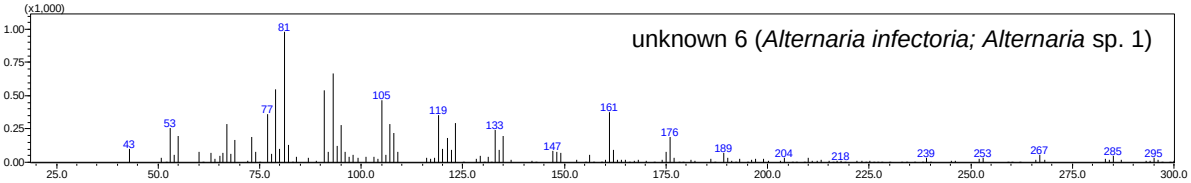
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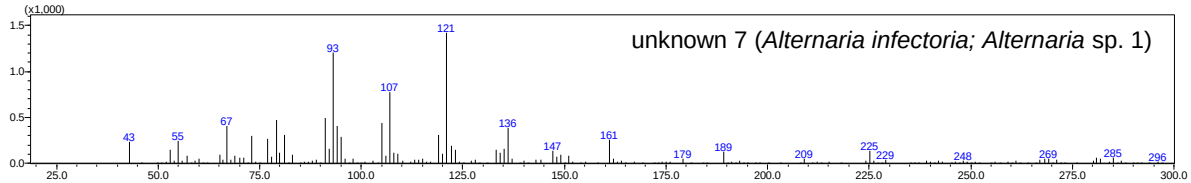
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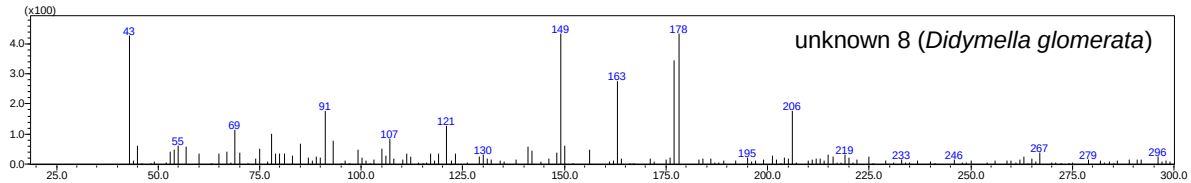
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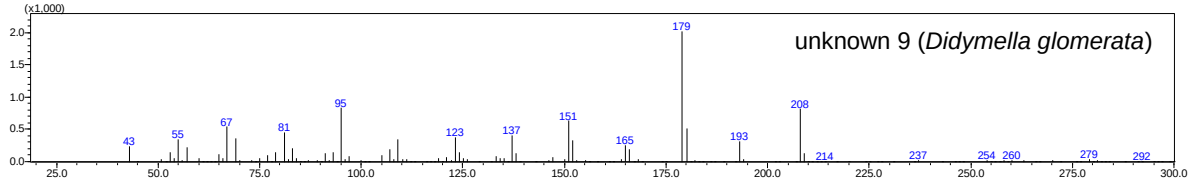
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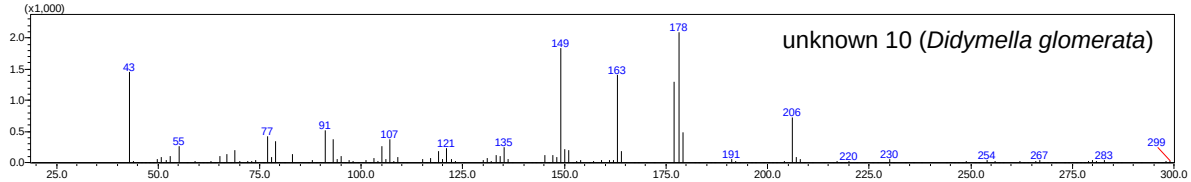
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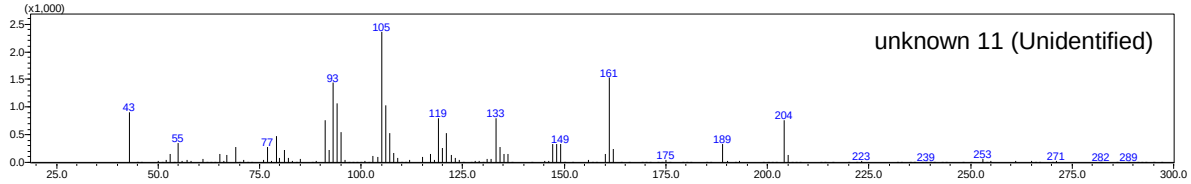
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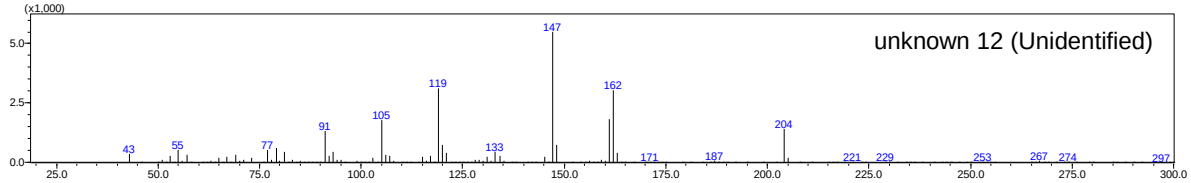
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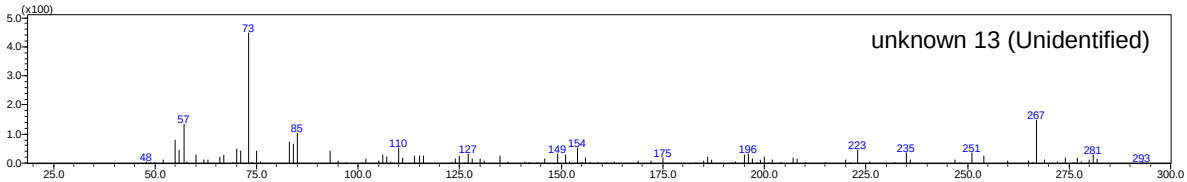
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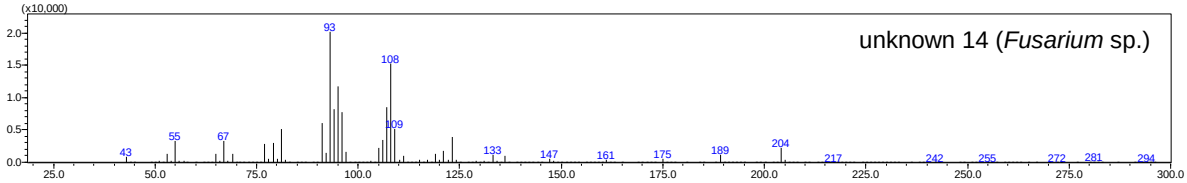
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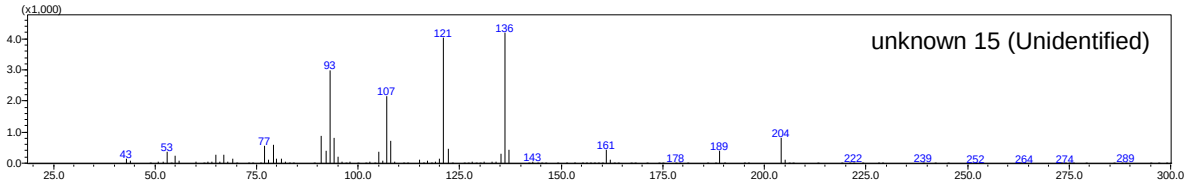
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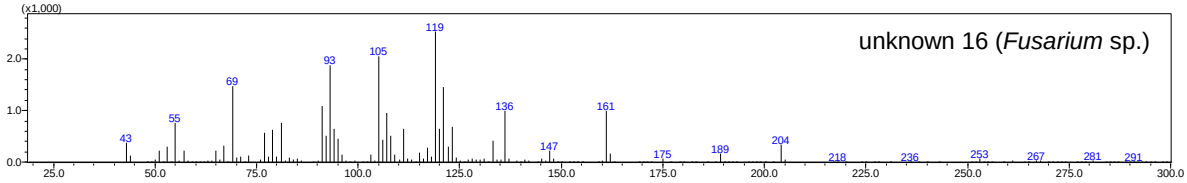
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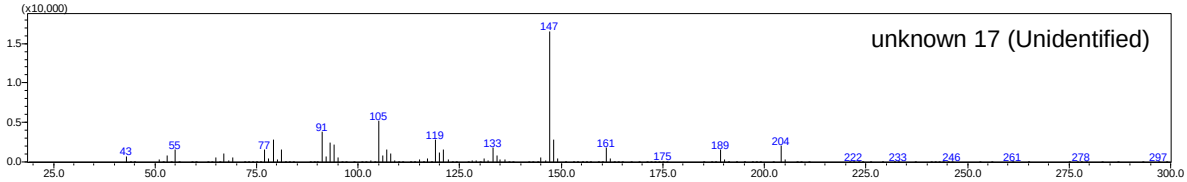
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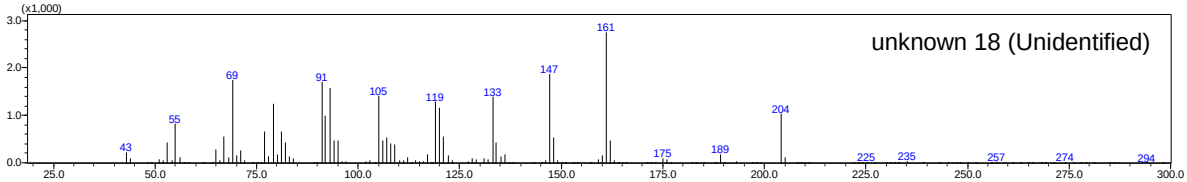
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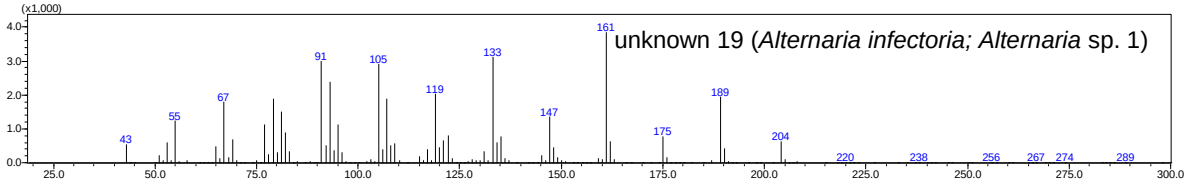
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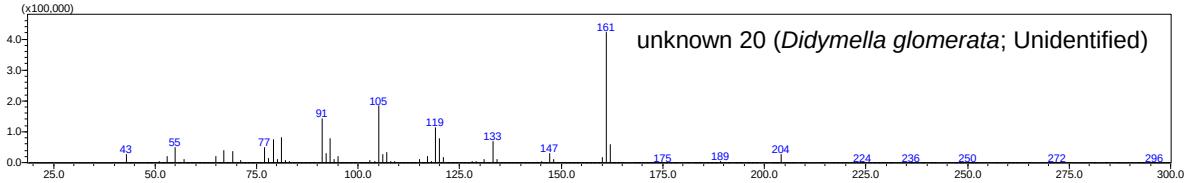
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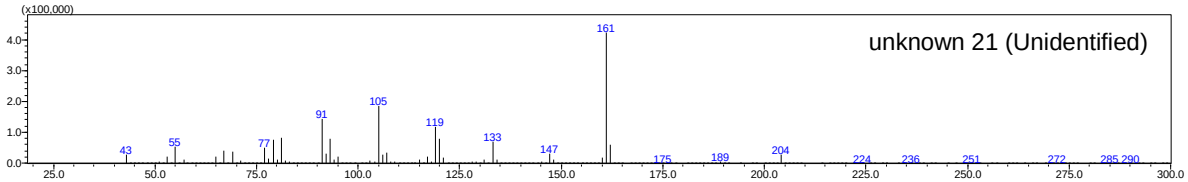
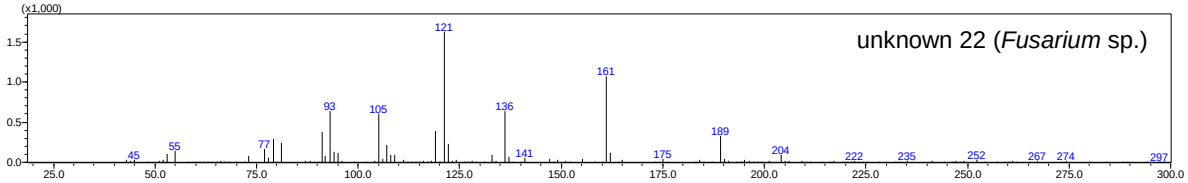
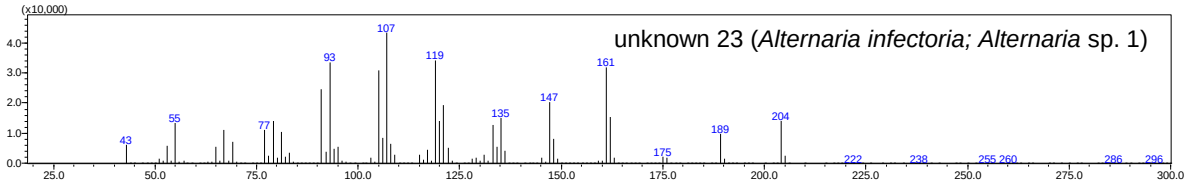
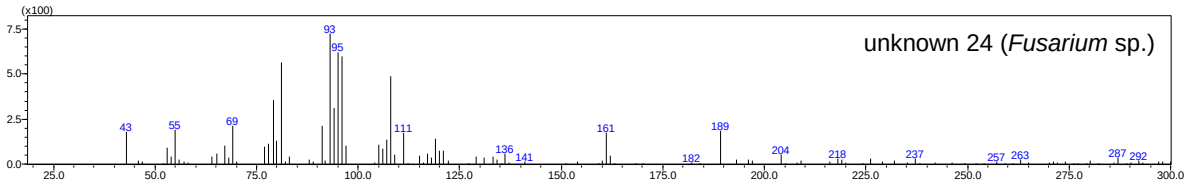
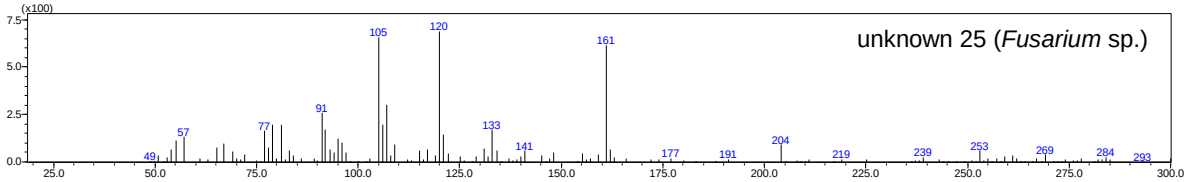
272
296


(x100,000)
unknown 21 (Unidentified)


(x1,000)
unknown 22 (*Fusarium* sp.)


(x10,000)
unknown 23 (*Alternaria infectoria; Alternaria* sp. 1)


(x100)
unknown 24 (*Fusarium* sp.)


(x100)
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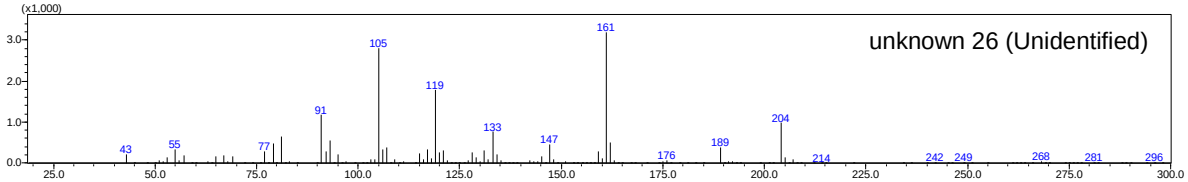
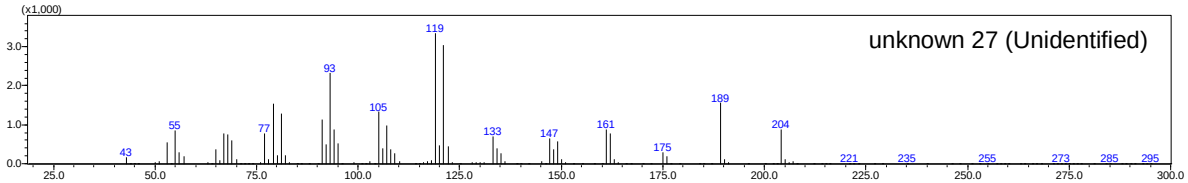
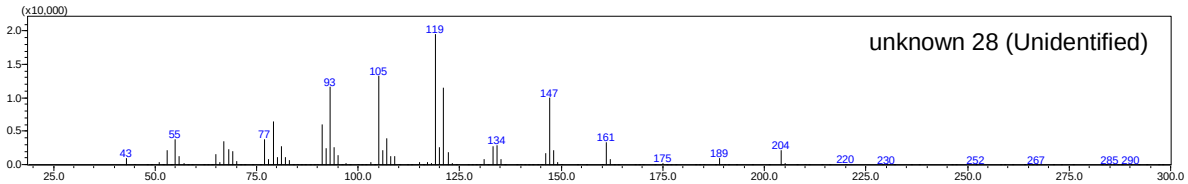

(x1,000)
unknown 26 (Unidentified)


(x1,000)
unknown 27 (Unidentified)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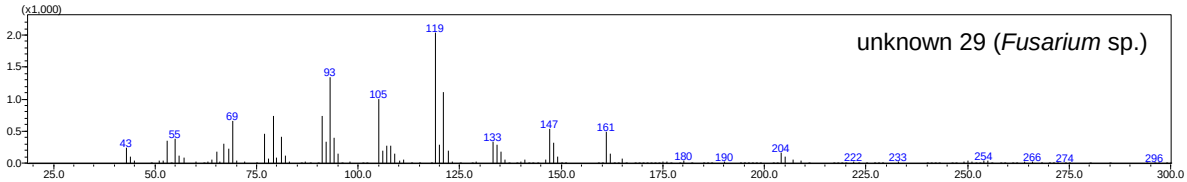
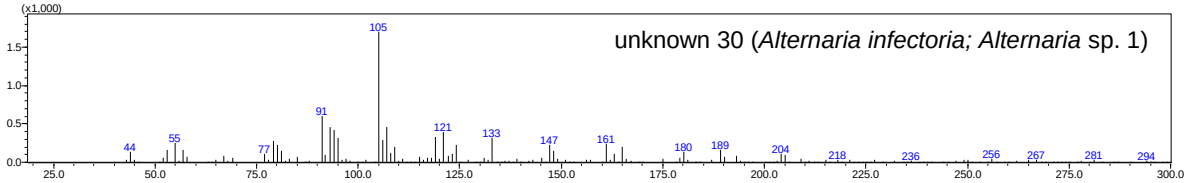
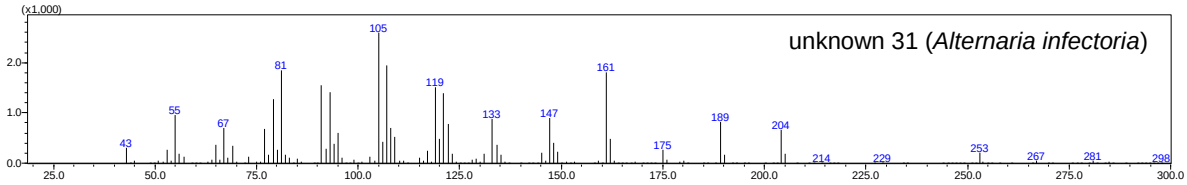
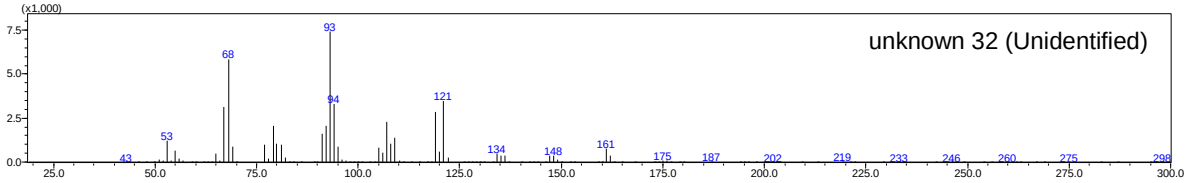
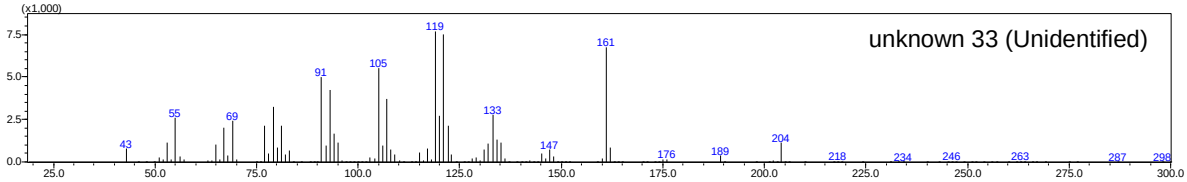
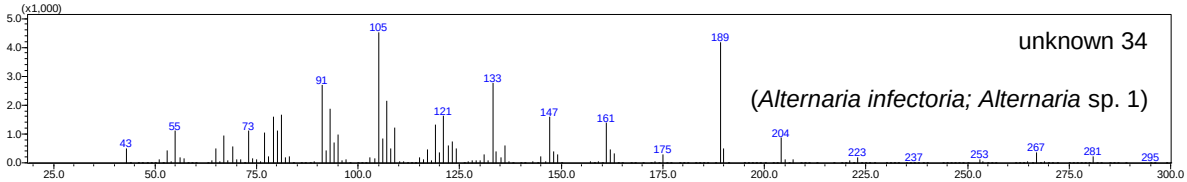

(x1,000)
unknown 29 (*Fusarium* sp.)

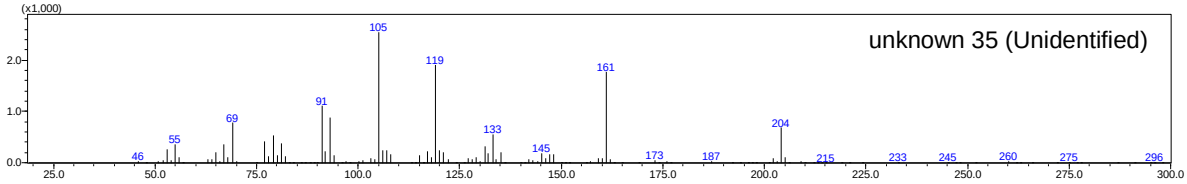

(x1,000)
unknown 30 (*Alternaria infectoria; Alternaria* sp. 1)

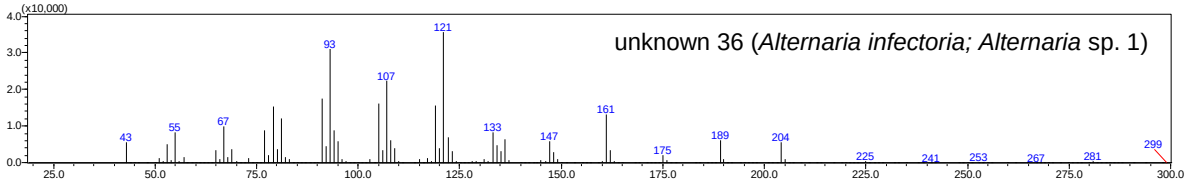

(x1,000)
unknown 31 (*Alternaria infectoria*)

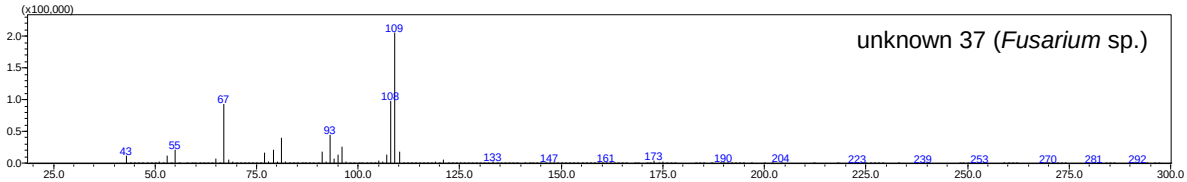

(x1,000)
unknown 32 (Unidentified)

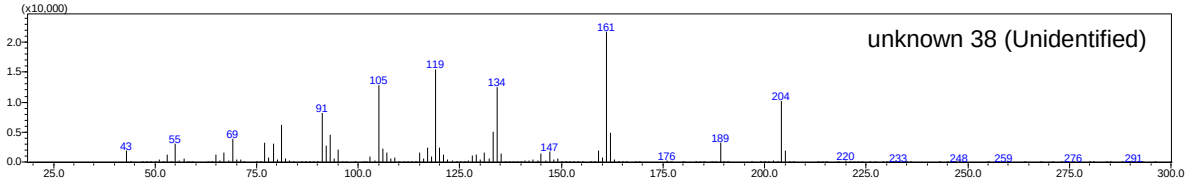

(x1,000)
unknown 33 (Unidentified)

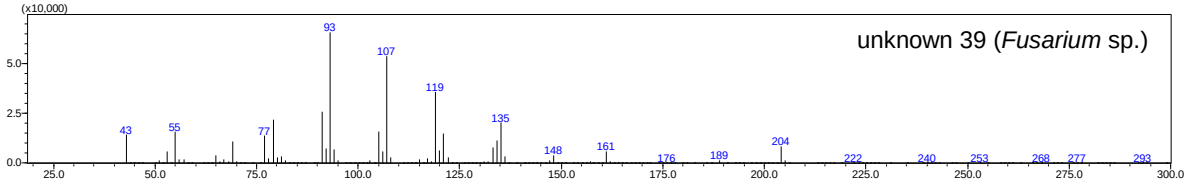

(x1,000)
unknown 34
(*Alternaria infectoria; Alternaria* sp. 1)

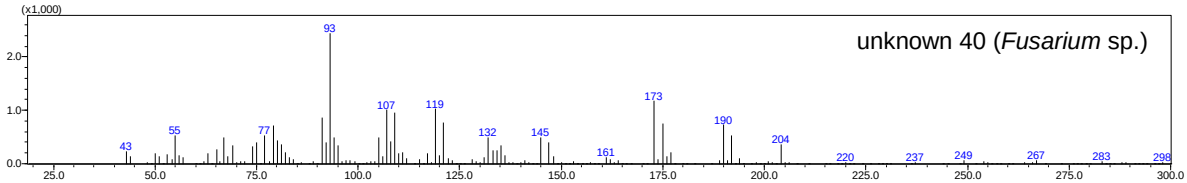

(x1,000)
unknown 35 (Unidentified)

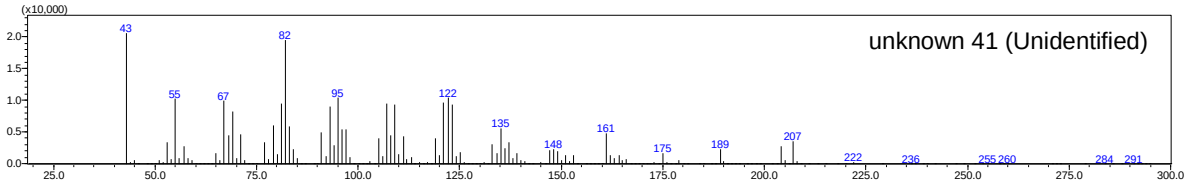

(x10,000)
unknown 36 (*Alternaria infectoria; Alternaria* sp. 1)

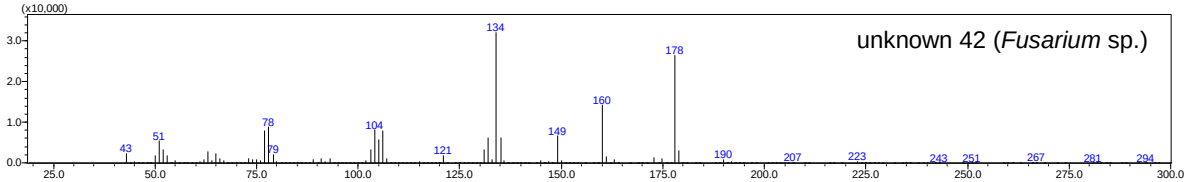

(x100,000)
unknown 37 (*Fusarium* sp.)
109
108
67
93
43
55
133
147
161
173
190
204
223
239
253
270
281
292

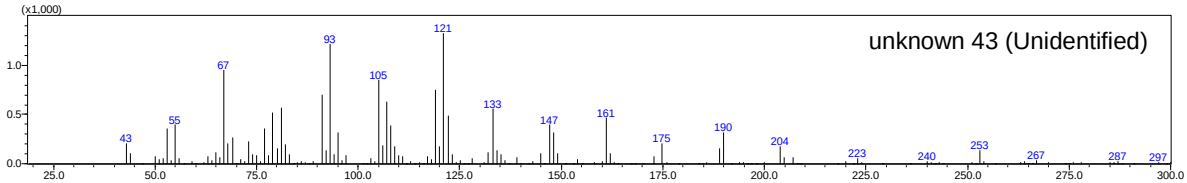

(x10,000)
unknown 38 (Unidentified)
161
119
105
134
91
204
43
55
69
147
189
176
220
233
248
259
276
291

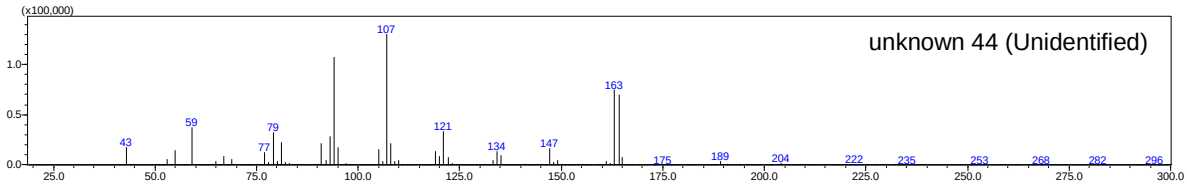

(x10,000)
unknown 39 (*Fusarium* sp.)
93
107
119
135
43
55
77
148
161
176
189
204
222
240
253
268
277
293


(x1,000)
unknown 40 (*Fusarium* sp.)
93
107
119
173
190
132
145
55
77
43
161
204
220
237
249
267
283
298


(x10,000)
unknown 41 (Unidentified)
43
82
55
67
95
122
135
161
148
175
189
207
222
236
255
260
284
291


(x10,000)
unknown 42 (*Fusarium* sp.)
134
178
160
78
104
149
51
43
79
121
190
207
223
243
251
267
281
294


(x1,000)
unknown 43 (Unidentified)
121
93
67
105
133
147
161
55
43
175
190
204
223
240
253
267
287
297


(x100,000)
unknown 44 (Unidentified)
107
163
59
79
121
43
77
134
147
175
189
204
222
235
253
268
282
296

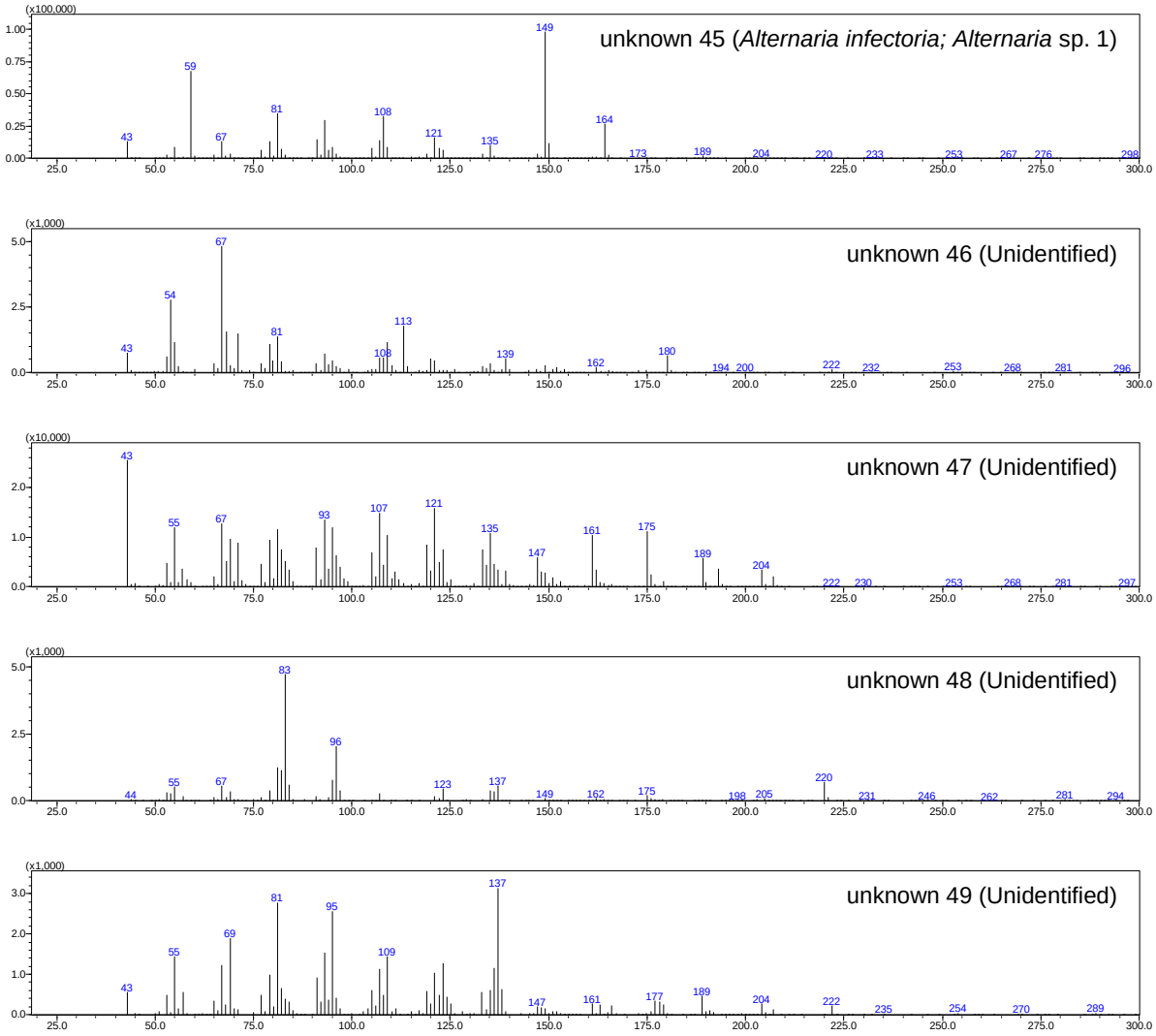


Figure S2: Mass spectra of unknown volatile organic compounds shown in Table 2. Background has been subtracted.

BUSCO version is: 3.0.2
The lineage dataset is: fungi_odb9 (Creation date: 2016-02-13, number of species: 85, number of BUSCOs: 290)
To reproduce this run: python /opt/software/bin/run_BUSCO.py -i /opt/software/packages/galaxy-dist/database/files/009/dataset_9623.dat -o busco_galaxy -l /opt/software/packages/busco/lineage/fungi_odb9/ -m transcriptome -c 11 -e 0.01 -z
#
Summarized benchmarking in BUSCO notation for file /opt/software/packages/galaxy-dist/database/files/009/dataset_9623.dat
BUSCO was run in mode: transcriptome

C:98.3%[S:93.1%,D:5.2%],F:1.4%,M:0.3%,n:290

285	Complete BUSCOs (C)
270	Complete and single-copy BUSCOs (S)
15	Complete and duplicated BUSCOs (D)
4	Fragmented BUSCOs (F)
1	Missing BUSCOs (M)
290	Total BUSCO groups searched

Figure S3: BUSCO analysis of the *Cladosporium* sp. *de novo* assembly. The BUSCO software tool [1] was used to validate the completeness of the *de novo* assembly.

Reference

1. Afgan, E.; Baker, D.; Batut, B.; Van Den Beek, M.; Bouvier, D.; Čech, M.; Chilton, J.; Clements, D.; Coraor, N.; Grüning, B. A., *Nucleic acids research* **2018,** *46* (W1), W537-W544.