Supporting Information

Imprint DESI-MS imaging monitors secondary metabolites production during antagonistic interaction of fungi.

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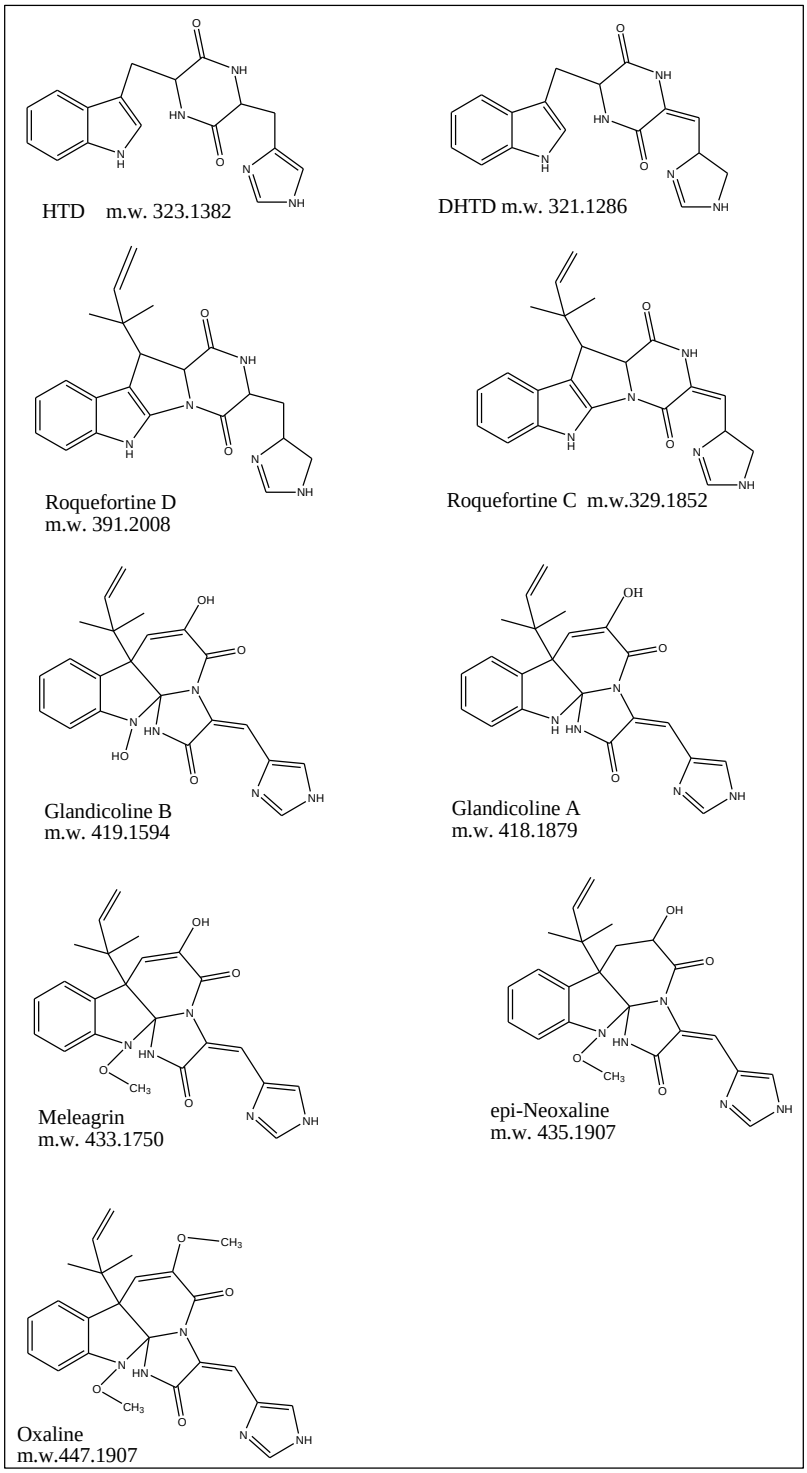

Figure S1. Metabolites observed in sporulating *M.roreri.* The chemical structures and the molecular weights (m.w.) of each metabolite are reported.

TableS1. List of metabolites detected in sporulating *M. roreri*. The m/z values detected during imprint-DESI-MS analysis and *via* LC-HRMS experiments, the assignment, the type of ion and the fragments observed both by DESI-MS and LC-MS/MS, and elemental formula of the ion species are reported.

m/z DESI-MS	m/z LC-HRMS	m/z theoretical	Error (ppm)	Assignment	Type of ion	MS/MS fragments	Elemental Formula
322.2	322.1303	322.1299	1.24	DHDT	$[M+H]^+$	277,193,162,130	$C_{17}H_{16}N_5O_2$
324.2	324.1461	324.1455	1.85	HDT	$[M+H]^+$	307,296,279,224,195,159,110	$C_{17}H_{18}N_5O_2$
390.2	390.1932	390.1925	1.79	Roquefortine C	$[M+H]^+$	334.322,198,193	$C_{22}H_{24}N_5O_2$
392.2	392.2080	392.2081	-0.25	Roquefortine D	$[M+H]^+$	336,324,195	$C_{22}H_{26}N_5O_2$
403.2	403.1638	403.1644	-1.48	Meleagrin-CH_3O	$[M+H]^+$	385, 336, 335	$C_{22}H_{21}N_5O_3$
404.2	404.1706	404.1717	-2.72	Glandicoline A	$[M+H]^+$	386, 335, 336,319	$C_{22}H_{22}N_5O_2$
418.2	418.1974	N/A	N/A	N/A	N/A	401,282,194,137	N/A
420.2	420.1668	420.1666	0.48	Glandicoline B	$[M+H]^+$	403,334,289	$C_{22}H_{22}N_5O_4$
434.2	434.1818	434.1823	-1.15	Meleagrin	$[M+H]^+$	403, 366,334	$C_{23}H_{24}N_5O_4$
436.2	436.1978	436.1984	1.38	epi-neoxaline	$[M+H]^+$	405, 368, 336	$C_{23}H_{26}N_5O_4$
448.2	448.1978	448.1985	-1.56	oxaline	$[M+H]^+$	430,404,388,336	$C_{24}H_{26}N_5O_4$

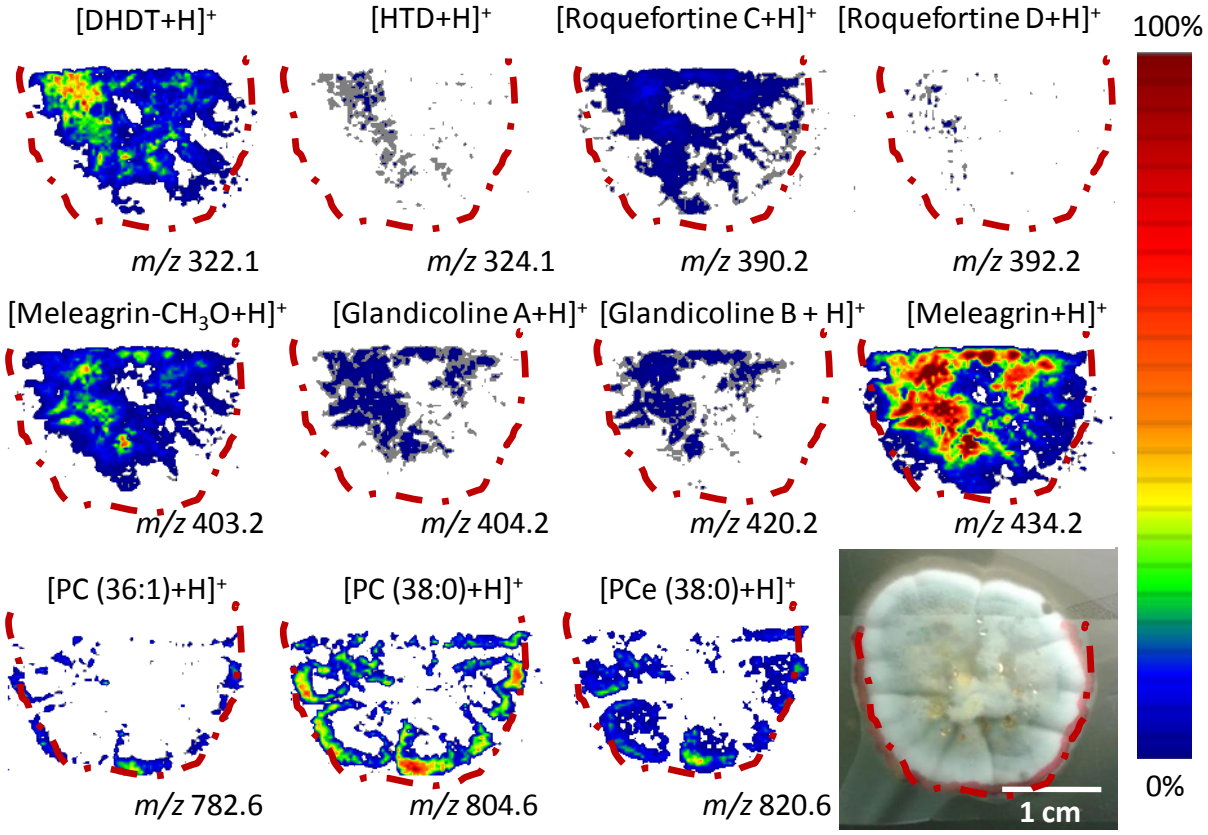

Figure S2. DESI-MS imaging of the colony. Interestingly, DHTD, HTD, roquefortine C, roquefortine D, meleagrin–CH_3O, glandicoline A, glandicoline B and meleagrine were observed in the center and left side where the yellow exudates are concentrated, whereas in the right, where the red patched with encrusted hyphes may be present, the same metabolites have a lower relative abundance. It is worth noticing that all the metabolites belonging to the same pathway have the same localization in the sample. In the white edge of the colony only phosphatidylcholines (PC) of *m/z*782.6, 804.6 and 820.6 were observed. The dashed outline indicates the imaged area.

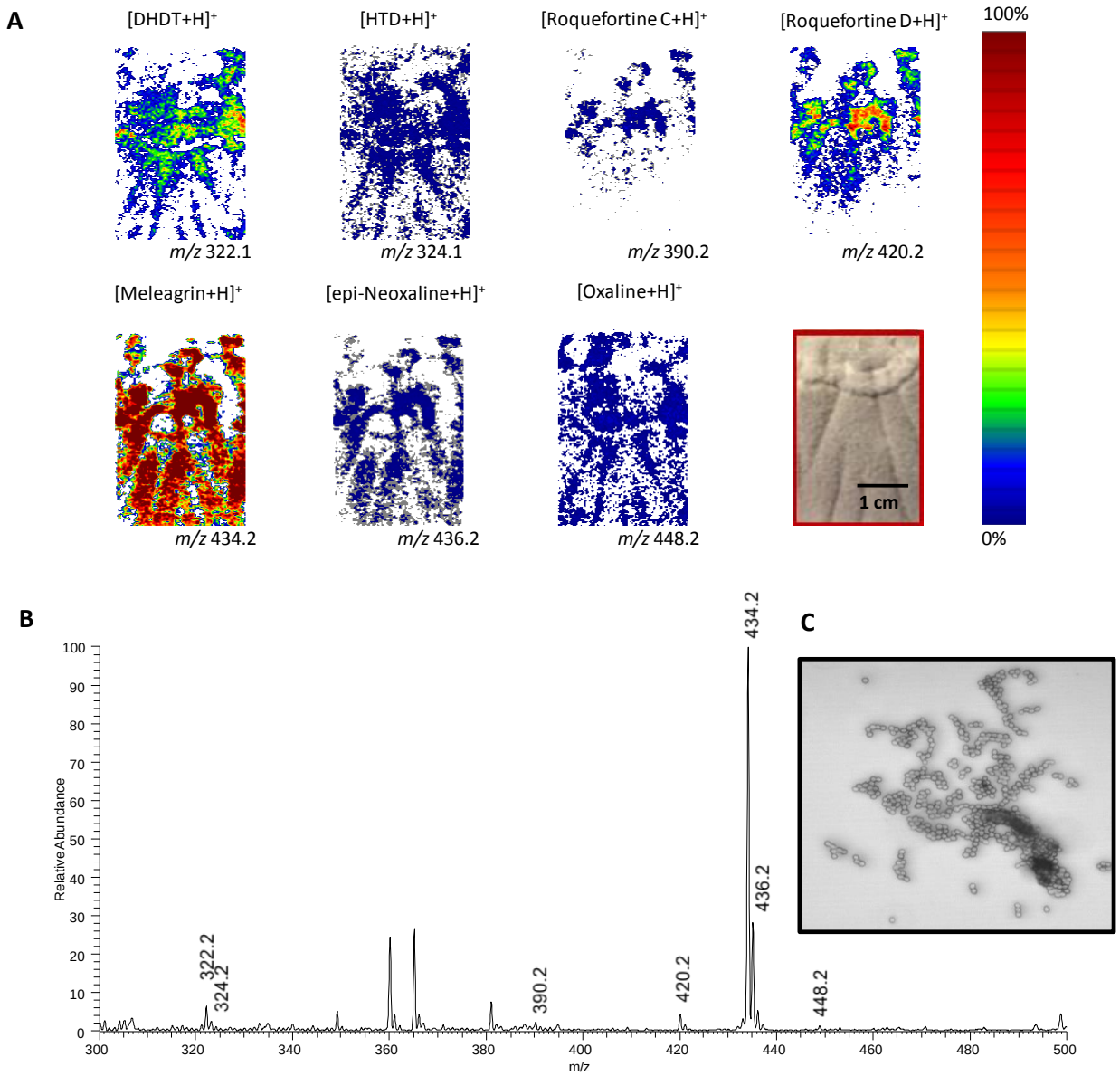

Figure S3 A)DESI-MS spectrum and B) DESI-MS imaging of the M. roreri metabolites observed during massive sporulation. C) Optical image of *M.roreri* spores.

Table S2. List of peptaibolic ions observed in their monoisotopic mass in *T.harzianum* monoculture and coculture. The assignment was done by tandem mass spectrometry and literature search *(S.Suwan et al. 2000, J.Mass Spectrom., 35,1438-1451 ; S.Rebuffat et al. 1995, J.Chem.Pekin Trans, 1 , 1849-1855)*

m/z DESI-MS	*Assignment*	*Type of ion*	*MS/MS fragments*
722.5	Harzianin VIII	$[M+2Na]^{++}$	1406,983,713,601,559,435,349,264,
726.5	Harzianin XII	$[M+H+Na]^{++}$	1412,718,605,576,506,483
730.5	Harzianin XI	$[M+2Na]^{++}$	1422,731,609,580,435,349,264
738.5	Harzianin X	$[M+K+Na]^{++}$	1436,729,616,602,448,363,278
744.5	Harzianin III	$[M+K+Na]^{++}$	1450,736,623,581,524,377
746.5	Harzianin XII	$[M+K+Na]^{++}$	1452,737.623,595,448,363,278
752.5	Harzianin XV	$[M+K+Na]^{++}$	1466,743,697,641,617,503,427,379
760.5	Harzianin XIII	$[M+2K]^{++}$	1482,742,673,648,535,480,283
864.5	Trichotoxin 1	$[M+K+H]^{++}$	855,740,649,536,426,341
871.5	Trichotoxin 2	$[M+K+H]^{++}$	1078,862,821,662,536,341
874.5	Trichotoxin 2	$[M+2Na]^{++}$	1745,1072,865,802,763,648
877.5	Trichotoxin 4	$[M+Na+H]^{++}$	869,764,577,544,1733
878.5	Trichotoxin 3	$[M+K+H]^{++}$	869,828.544,440,325
881.5	Trichotoxin 3	$[M+2Na]^{++}$	1725,968,872,809,702,662,633,548
888.5	Trichotoxin 4	$[M+2Na]^{++}$	1740,879,775,663
889.5	Trichotoxin 3	$[M+K+H]^{++}$	1739,1666,1414,1102,1002,880,816,880,816,662,647,590
896.5	Trichotoxin 4	$[M+K+Na]^{++}$	1754,1101,885,823,662,562
903.5	Trichotoxin 5	$[M+K+Na]^{++}$	1768,847,791,773,648,545,487
1169.8	N/A	N/A	1151,1113,955,927,870,842,729,659,617,545
1183.9	N/A	N/A	1165,1127,941,884,856,743,631,522
1197.8	N/A	N/A	1179,1141,1085,983,955,870,898757,631
1409.0	Harzianin VI	$[M+Na]^{+}$	1166,1109,1081,969,786,701,617
1423.0	Harzianin VIII	$[M+Na]^{+}$	1404,1208,1180,1123,1095,983,800,715,617,602
1437.0	Harzianin IX	$[M+Na]^{+}$	1420,1223,1196,1138,1111,999,816,731,617,559
1439.0	Harzianin VIII	$[M+K]^{+}$	1382,1327,1216,1198
1453.0	Harzianin IX	$[M+K]^{+}$	1434,1238,1210,1153,1125,1013,816,731
1465.0			1408,1250,1222,1132,1093,997

1467.0	Harzianin XV	$[M+K]^+$	1148,1252,1224,1167,1139,1013,830,745.647
1469.0	Harzianin XII	$[M+K]^+$	1451,1254,1226,1169,1141,955
1483.0	Harzianin XIII	$[M+K]^+$	1464,1426,1369,1310,1268,1240,1212,1111,1029
1726.1	Trichotoxin II	$[M+Na]^+$	1708,1609,1581,1453,1368,1283,1173,1072,987,973,903,817,732,648,576
1728.1	Trichotoxin I	$[M+K]^+$	1671,1616,1446,1350,1205,1048
1732.1	Trichotoxin IV	$[M+H]^+$	1675,1619,1537,1388
1740.1	Trichotoxin III	$[M+Na]^+$	1722,1683,1628,1073
1742.1	Trichotoxin II	$[M+K]^+$	1685,1630,1509,1106
1754.1	Trichotoxin IV	$[M+Na]^+$	1737,1637,1609,1481,1382,1296,1086,1001,917,831, 746,662
1768.1	Trichotoxin V	$[M+Na]^+$	1752,1652,1624,1496,1397,1312,1102,1017,933,847,762,662,562
1770.1	Trichotoxin IV	$[M+K]^+$	1713,1658,1602,1583,1469

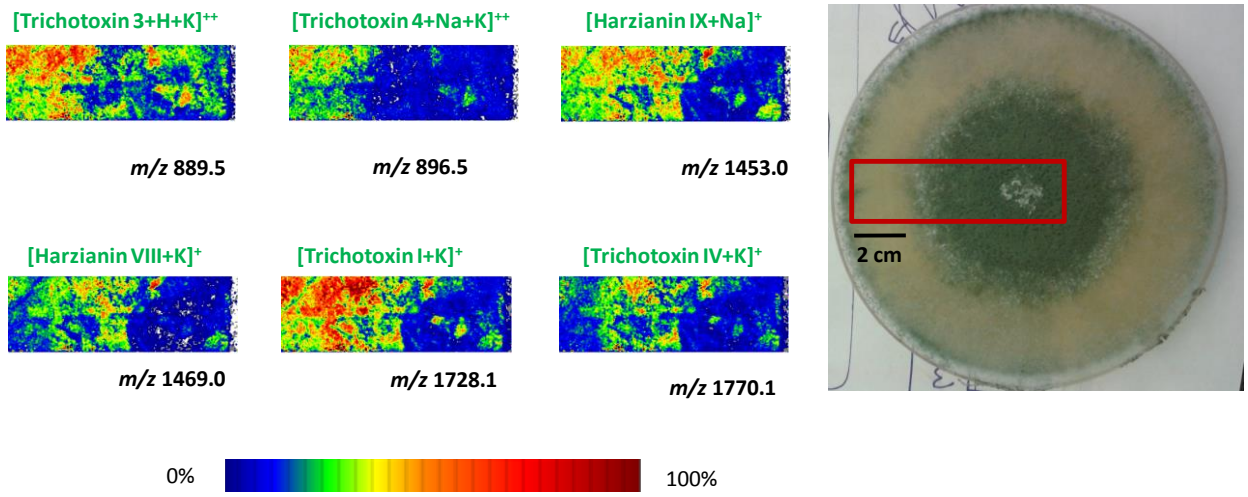

Figure S4. DESI-MS imaging of the *T.harzianum* metabolites observed during massive sporulation. C) Optical image T.harzianum plate after two weeks from the inoculation.

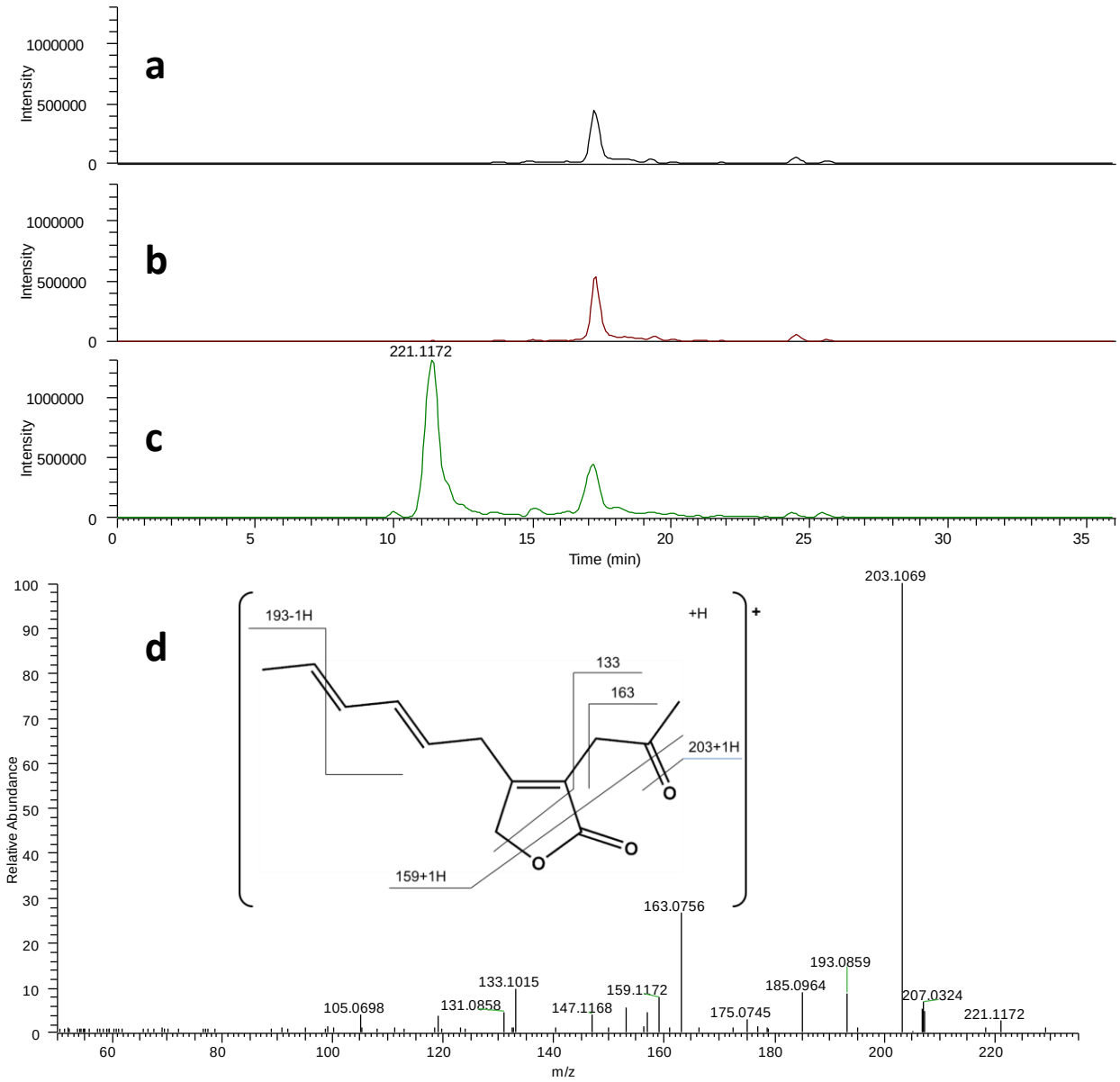

Figure S5. LC-HMRS separation of the secondary metabolite T39butenolide of *m/z* 221.1172 from the extract of a) agar, b) *T.harzianum* colony and c) coculture. The comparison of the three chromatograms reveals the exclusive production of the metabolite in the coculture at 11.2 min. In both monocultures and agar extracts, the secondary metabolite of *m/z* 221.1172 was not observed. d) Fragmentation of the secondary metabolite T39butenolide of *m/z* 221.1172. The fragments observed by LC-HRMS/MS at 11.2 min are in accordance with the proposed structure and literature (Vinale *et. al.* 2009).

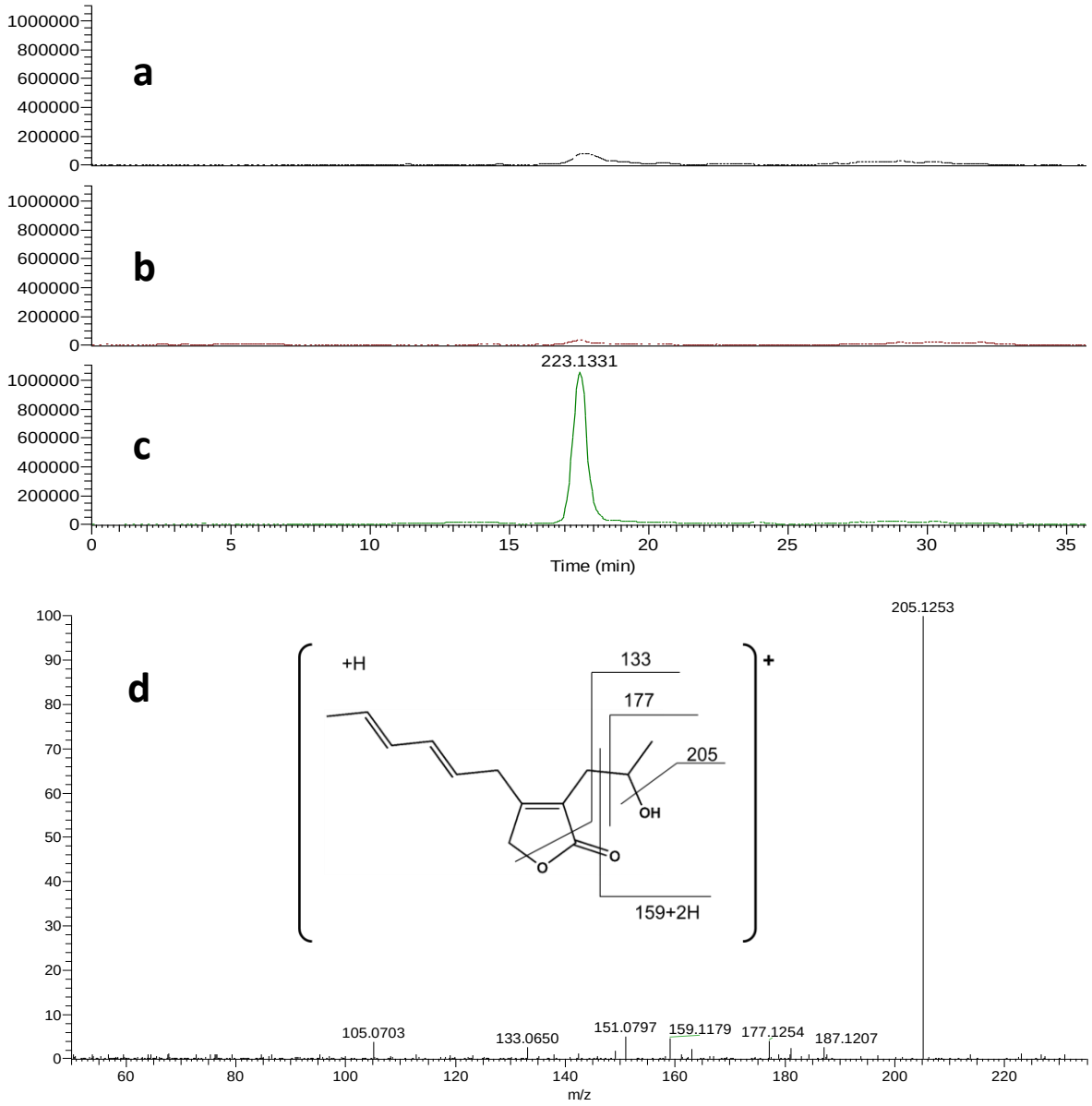

Figure S6. LC-HMRS separation of the secondary metabolite harzianolide of *m/z* 223.1331 from the extract of a) agar, b) *T.harzianum* colony and c) coculture. The comparison of the three chromatograms reveals the almost exclusive production of the metabolite in the coculture at 17.5 min. In both monocultures and agar extracts, the secondary metabolite of *m/z* 223.1331 was not observed. d) Fragmentation of the secondary metabolite harzianolide of *m/z* 223.1331. The fragments observed by LC-HRMS/MS at 17.5 min are in accordance with the proposed structure and literature. (Vinale *et. al.* 2009)

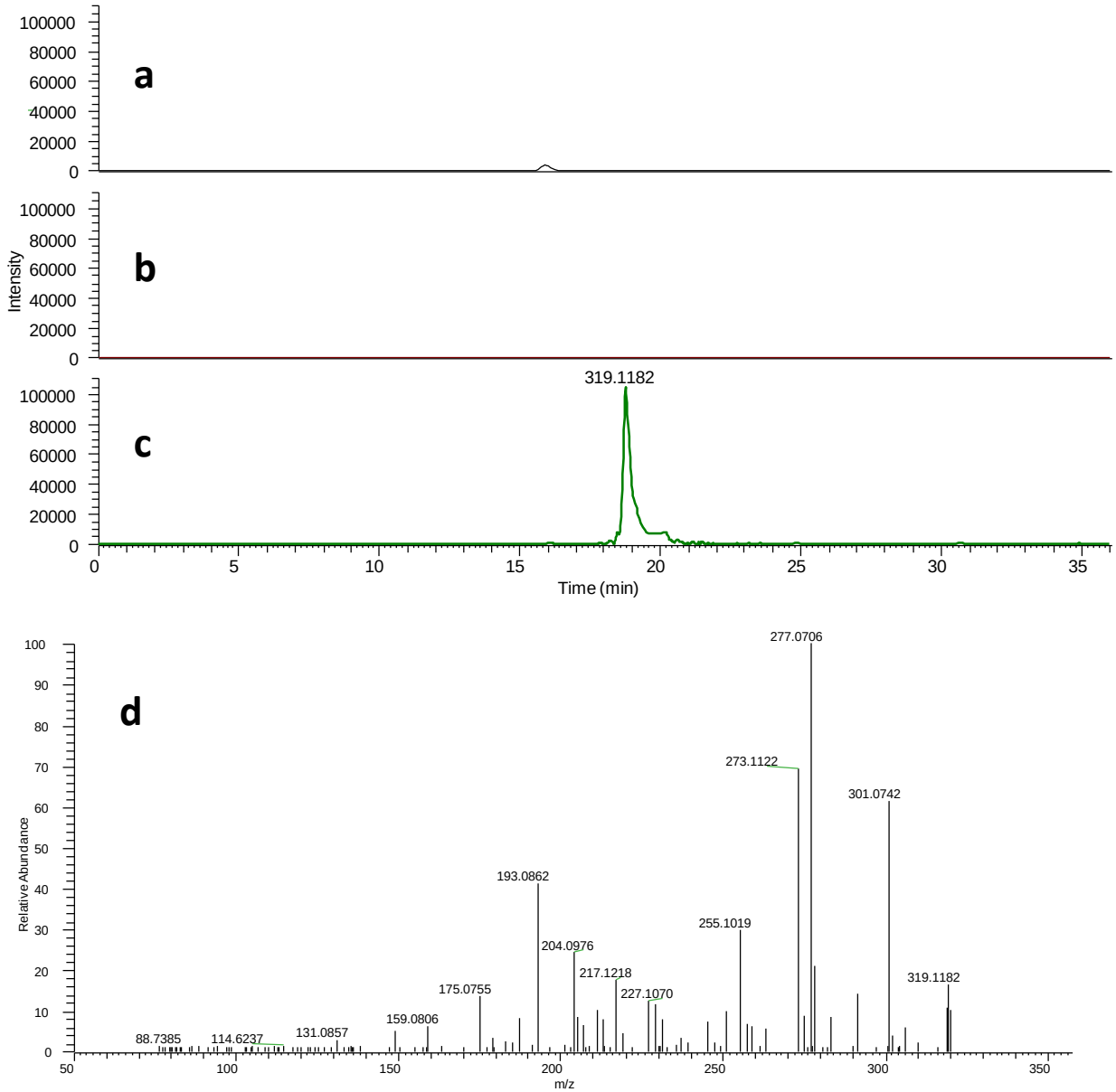

Figure S7 . LC-HMRS separation of the secondary metabolite *m/z* 319.1182 from the extract of a) agar, b) *T.harzianum* colony and c) coculture. The comparison of the three chromatograms reveals the exclusive production of the metabolite in the coculture at 18.8 min. In both monocultures and agar extracts, the secondary metabolite *m/z* 319.1182 was not observed. d) Fragmentation of the secondary metabolite *m/z* 319.1182.

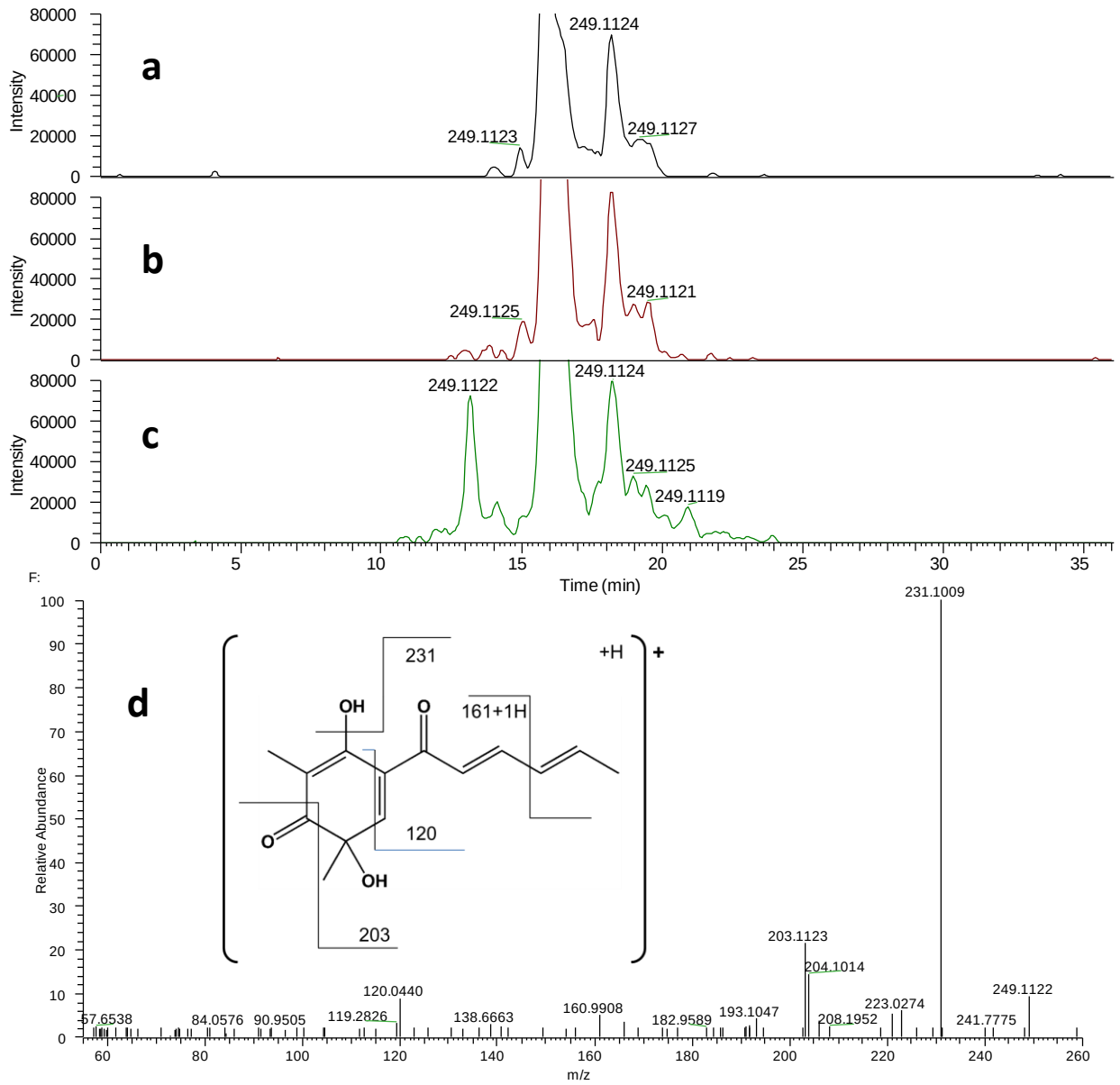

Figure S8. LC-HMRS separation of the secondary metabolite sorbicillino of *m/z* 249.1122 from the extract of a) agar, b) *T.harzianum* colony and c) coculture. The comparison of the three chromatograms reveals the exclusive production of the metabolite in the coculture at 13.2 min. In both monocultures and agar extracts, the secondary metabolite sorbicillinol of *m/z* 249.1122 were not observed. d) Fragmentation of the secondary metabolite sorbicillinol of *m/z* 249.1122. The fragments observed by LC-HRMS/MS at 13.2 min are in accordance with the proposed structure.