

Metabolic plant responses to above- and belowground herbivory and plant-mediated crosstalk between herbivores on *Arabidopsis thaliana*

Magdalene Kutyniok and Caroline Mueller

Table S1. Mean relative abundances (\pm SD) of primary metabolites identified by GC-MS in *Arabidopsis thaliana* shoot tissue of control (C), aphid (A), nematode (N) and aphid+nematode treated plants (AN) 3 dpi. The substance identities were either verified by comparison with standards, with help of the online Golm database (GMD) using spectra and Kovats indices, or could not be verified. There were ten replicates per treatment; data are shown from one representative experiment out of two independent experiments. Metabolites that differed significantly between treatments are indicated in bold (Kruskal-Wallis, $P < 0.05$). However, after Bonferroni correction, there were no significant differences between treatments ($P > 0.05$).

Substance	Kovats index	Standard	GMD	Not identified	C	A	N	AN
Pyruvic acid	1038	X	X		0.0047 (0.0009)	0.0044 (0.0006)	0.0051 (0.0012)	0.0041 (0.0008)
Proline	1181	X	X		0.0068 (0.0066)	0.0129 (0.0086)	0.0367 (0.0360)	0.0077 (0.0068)
Valine	1209	X	X		0.0030 (0.0016)	0.0037 (0.0013)	0.0035 (0.0013)	0.0033 (0.0026)
Serine	1252/1353	X	X		0.177 (0.063)	0.181 (0.045)	0.184 (0.031)	0.201 (0.079)
Ethanolamine	1260		X		0.0088 (0.0014)	0.0095 (0.0041)	0.0111 (0.0025)	0.0106 (0.0024)
Phosphoric acid	1263		X		0.0167 (0.004)	0.015 (0.003)	0.013 (0.004)	0.015 (0.002)
Glycerol	1266	X	X		1.365 (0.261)	1.532 (0.261)	1.485 (0.446)	1.473 (0.207)
Threonine	1289/1378	X	X		0.065 (0.024)	0.072 (0.013)	0.069 (0.017)	0.072 (0.021)
Glycine	1300	X	X		0.048 (0.021)	0.051 (0.015)	0.043 (0.014)	0.039 (0.010)
Succinic acid	1310	X	X		0.046 (0.015)	0.037 (0.020)	0.040 (0.012)	0.041 (0.018)
Glyceric acid	1323		X		0.021 (0.004)	0.017 (0.004)	0.019 (0.003)	0.016 (0.003)
unknown	1327			X	0.0067 (0.0027)	0.0084 (0.0043)	0.0159 (0.1900)	0.0169 (0.0142)
Fumaric acid	1347	X	X		8.081 (5.538)	4.565 (4.261)	5.625 (3.338)	4.281 (3.472)

Threonic acid-1,4-lactone	1370		X	0.0099 (0.0032)	0.0077 (0.0013)	0.0081 (0.0013)	0.0085 (0.0029)
Aspartic acid	1421/1514		X	0.063 (0.015)	0.066 (0.018)	0.063 (0.017)	0.073 (0.021)
Malic acid	1482	X	X	0.813 (0.180)	0.659 (0.175)	0.739 (0.187)	0.734 (0.404)
Pyroglutamic acid	1522		X	0.145 (0.026)	0.175 (0.038)	0.168 (0.049)	0.177 (0.038)
Butanoic acid, 4-amino-	1524	X	X	0.0070 (0.0041)	0.0075 (0.0072)	0.0083 (0.0045)	0.0093 (0.0055)
Glutamic acid	1529/1614	X	X	0.060 (0.024)	0.060 (0.026)	0.059 (0.016)	0.064 (0.046)
unknown	1551		X	0.084 (0.022)	0.044 (0.028)	0.057 (0.031)	0.074 (0.043)
unknown	1580		X	0.0082 (0.0033)	0.0100 (0.0049)	0.0106 (0.0039)	0.0131 (0.0068)
unknown	1594		X	0.0087 (0.0037)	0.0092 (0.0067)	0.0124 (0.0041)	0.0149 (0.0073)
unknown	1634		X	0.013 (0.003)	0.013 (0.005)	0.014 (0.002)	0.015 (0.003)
Glycerol-3-phosphate	1752	X	X	0.0105 (0.0065)	0.0067 (0.0043)	0.0092 (0.0055)	0.0084 (0.0082)
Shikimic acid	1801	X	X	0.0057 (0.0032)	0.0066 (0.0025)	0.0053 (0.0031)	0.0047 (0.0033)
Citric acid	1809	X	X	0.257 (0.071)	0.263 (0.040)	0.241 (0.061)	0.264 (0.058)
Dehydroascorbic acid	1840/1865		X	0.280 (0.070)	0.227 (0.120)	0.270 (0.039)	0.268 (0.100)
Fructose	1859/1868	X	X	1.682 (1.235)	0.895 (0.746)	1.058 (0.788)	1.025 (0.886)
Galactose	1877	X	X	0.0092 (0.0064)	0.0057 (0.0035)	0.0073 (0.0029)	0.0062 (0.0041)
Glucose	1884/1903	X	X	2.291 (2.020)	1.112 (1.013)	1.589 (0.864)	1.160 (0.842)
unknown	1896		X	0.066 (0.050)	0.035 (0.033)	0.047 (0.019)	0.042 (0.027)
unknown	1906		X	1.039 (0.953)	0.327 (0.322)	0.637 (0.333)	0.594 (0.488)
unknown	1925		X	0.0100 (0.0015)	0.0114 (0.0035)	0.0105 (0.0030)	0.0123 (0.0033)
unknown	1930		X	0.107 (0.014)	0.098 (0.025)	0.100 (0.023)	0.107 (0.015)

unknown	1939		X	0.240 (0.059)	0.188 (0.074)	0.194 (0.052)	0.203 (0.061)
Gluconic acid	1952		X	0.0058 (0.0005)	0.0054 (0.0018)	0.0053 (0.0014)	0.0060 (0.0013)
Galatonic acid	1982		X	0.026 (0.017)	0.018 (0.007)	0.021 (0.006)	0.021 (0.008)
unknown	2031		X	0.0096 (0.0026)	0.0102 (0.0023)	0.0102 (0.0019)	0.0111 (0.0031)
unknown	2068		X	0.025 (0.006)	0.022 (0.006)	0.021 (0.007)	0.024 (0.005)
Inositol, myo-	2079	X	X	2.314 (0.905)	1.670 (0.704)	1.909 (0.466)	1.745 (0.563)
unknown	2109		X	0.0066 (0.0031)	0.0080 (0.0043)	0.0064 (0.0031)	0.0072 (0.0046)
Phytol	2169		X	0.0133 (0.0044)	0.0095 (0.0038)	0.0111 (0.0029)	0.0102 (0.0043)
unknown	2175		X	0.013 (0.003)	0.015 (0.009)	0.013 (0.003)	0.012 (0.005)
unknown	2215		X	0.159 (0.031)	0.169 (0.023)	0.152 (0.020)	0.172 (0.027)
Sucrose	2615	X		3.939 (0.840)	3.614 (1.135)	3.513 (0.648)	3.783 (0.590)
Trehalose, α - α	2722		X	0.0084 (0.0024)	0.0289 (0.0561)	0.0080 (0.0022)	0.0628 (0.1320)
unknown	2953		X	0.070 (0.013)	0.071 (0.009)	0.067 (0.013)	0.071 (0.013)
Galactinol	2963		X	0.028 (0.016)	0.017 (0.013)	0.023 (0.011)	0.023 (0.017)
unknown	2984		X	0.078 (0.011)	0.078 (0.016)	0.084 (0.012)	0.093 (0.018)
unknown	2996		X	0.012 (0.003)	0.013 (0.002)	0.011 (0.003)	0.012 (0.003)
unknown	3026		X	0.366 (0.098)	0.392 (0.122)	0.385 (0.116)	0.396 (0.120)
unknown	3046		X	0.0062 (0.0021)	0.0067 (0.0025)	0.0069 (0.0012)	0.0075 (0.0023)
unknown	3103		X	0.0092 (0.0074)	0.0083 (0.0071)	0.0096 (0.0074)	0.0083 (0.0082)
Raffinose	3354		X	0.144 (0.080)	0.108 (0.083)	0.122 (0.051)	0.140 (0.083)

Table S2. Mean values (\pm SD) of shoot and root glucosinolate and glucosinolate group concentrations (in μmol per g fresh weight) in control (C), aphid (A), nematode (N) and aphid+nematode treated plants (AN) 3 dpi. There were ten replicates per treatment; data are shown from one representative experiment out of three independent experiments. Lower case letters indicate significant differences between treatments (glm).

Tissue	Shoot				Root			
	C	A	N	AN	C	A	N	AN
Glucosinolate								
3-Methylthiopropyl	0.0034 (0.0011)	0.0021 (0.0012)	0.0032 (0.0021)	0.0230 (0.0009)		n.d.		
4-Methylthiobutyl	0.900 _a (0.127)	0.669 _b (0.178)	0.792 _a (0.234)	0.721 _b (0.178)	0.028 (0.007)	0.019 (0.008)	0.024 (0.012)	0.025 (0.012)
5-Methylthiopentyl	0.024 _a (0.005)	0.019 _b (0.004)	0.022 _a (0.006)	0.020 _b (0.005)	0.010 (0.030)	0.009 (0.002)	0.008 (0.002)	0.009 (0.002)
6-Methylthiohexyl	0.0045 (0.0012)	0.0035 (0.0010)	0.0042 (0.0015)	0.0035 (0.0010)		n.d.		
7-Methylthioheptyl	0.077 (0.022)	0.068 (0.022)	0.073 (0.020)	0.060 (0.014)	0.157 (0.068)	0.162 (0.039)	0.145 (0.065)	0.135 (0.039)
8-Methylthiooctyl	0.660 (0.097)	0.587 (0.153)	0.637 (0.137)	0.571 (0.120)	2.208 (0.563)	2.245 (0.356)	2.017 (0.611)	2.153 (0.460)
3-Methylsulfinylpropyl	0.135 _a (0.053)	0.085 _b (0.025)	0.111 _b (0.044)	0.099 _b (0.029)	0.021 _a (0.007)	0.013 _b (0.006)	0.019 _{ab} (0.007)	0.020 _a (0.007)
4-Methylsulfinylbutyl	1.389 _a (0.492)	0.907 _b (0.286)	1.143 _a (0.502)	1.026 _b (0.315)	0.227 _a (0.085)	0.134 _b (0.067)	0.204 _a (0.088)	0.227 _a (0.089)
5-Methylsulfinylpentyl	0.034 _a (0.010)	0.023 _b (0.005)	0.029 _a (0.010)	0.027 _a (0.007)	0.008 _a (0.002)	0.006 _b (0.002)	0.007 _a (0.002)	0.008 _a (0.02)

6-Methylsulfinyloxy	0.005 6 (0.0023)	0.003 4 (0.0014)	0.004 7 (0.0023)	0.003 7 (0.0013)	0.000 95 ^a (0.00048)	0.000 63 ^a (0.00022)	0.000 69 ^b (0.00027)	0.000 65 ^a (0.00030)
7-Methylsulfinyloxy	0.053 (0.015)	0.037 (0.013)	0.046 (0.017)	0.039 (0.010)	0.014 (0.005)	0.011 (0.003)	0.012 (0.004)	0.011 (0.004)
8-Methylsulfinyloxy	1.377 (0.290)	1.046 (0.325)	1.232 (0.399)	1.124 (0.257)	0.280 (0.081)	0.235 (0.059)	0.233 (0.057)	0.240 (0.064)
3-Methylbutyl	0.002 3 ^a (0.0006)	0.001 5 ^b (0.0004)	0.002 0 ^{ab} (0.0009)	0.001 8 ^{ab} (0.0005)			n.d.	
4-Methylpentyl	0.008 0 ^a (0.0020)	0.005 4 ^b (0.0014)	0.006 9 ^{ab} (0.0029)	0.006 3 ^{ab} (0.0018)	0.000 55 (0.00022)	0.000 39 (0.00020)	0.000 48 (0.00019)	0.000 54 (0.00019)
2-Phenethyl	0.018 a (0.005)	0.013 ^b (0.003)	0.016 ^a b (0.005)	0.015 ^a b (0.004)	0.000 9 (0.0004)	0.000 64 (0.0004)	0.000 8 (0.0003)	0.001 (0.0004)
Indol-3-ylmethyl	0.612 (0.205)	0.506 (0.173)	0.562 (0.161)	0.548 (0.160)	0.272 (0.032)	0.273 (0.064)	0.248 (0.071)	0.273 (0.072)
1-Methoxyindol-3-ylmethyl	0.069 (0.017)	0.072 (0.040)	0.099 (0.061)	0.084 (0.037)	1.589 (0.215)	1.341 (0.339)	1.576 (0.380)	1.639 (0.266)
4-Methoxyindol-3-ylmethyl	0.404 (0.132)	0.350 (0.082)	0.354 (0.102)	0.330 (0.082)	0.341 (0.082)	0.340 (0.049)	0.302 (0.086)	0.326 (0.054)
4-Hydroxyindol-3-ylmethyl	0.014 (0.005)	0.012 (0.003)	0.012 (0.004)	0.012 (0.004)	0.156 (0.037)	0.154 (0.036)	0.146 (0.056)	0.146 (0.028)
Total aliphatic	4.674 a (0.902)	3.457 ^b (0.844)	4.106 ^a b (1.232)	3.703 ^b (0.855)	2.956 (0.615)	2.835 (0.477)	2.672 (0.679)	2.830 (0.536)
Total indole	1.099 (0.303)	0.940 (0.252)	1.028 (0.258)	0.973 (0.241)	2.359 (0.333)	2.115 (0.408)	2.272 (0.557)	2.384 (0.364)
Total	5.790 (1.166)	4.409 (1.044)	5.150 (1.484)	4.691 (1.079)	5.316 (0.928)	4.951 (0.871)	4.945 (1.208)	5.215 (0.871)

n.d. not detected