

1 **New Phytologist Supporting Information**

2 **Article title:** *TOC1* in *Nicotiana attenuata* regulates efficient allocation of nitrogen
3 to defense metabolites under herbivory stress

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6 **Article acceptance date:** 16 June 2020

7

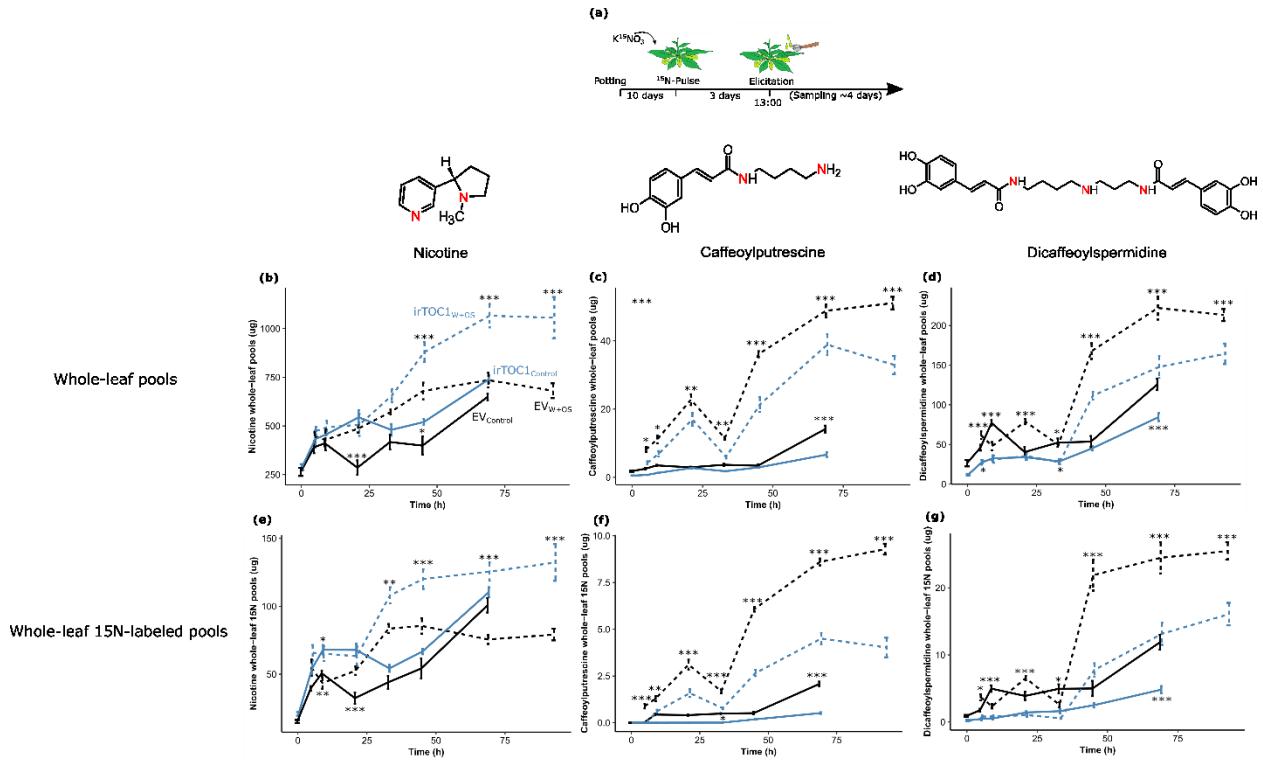
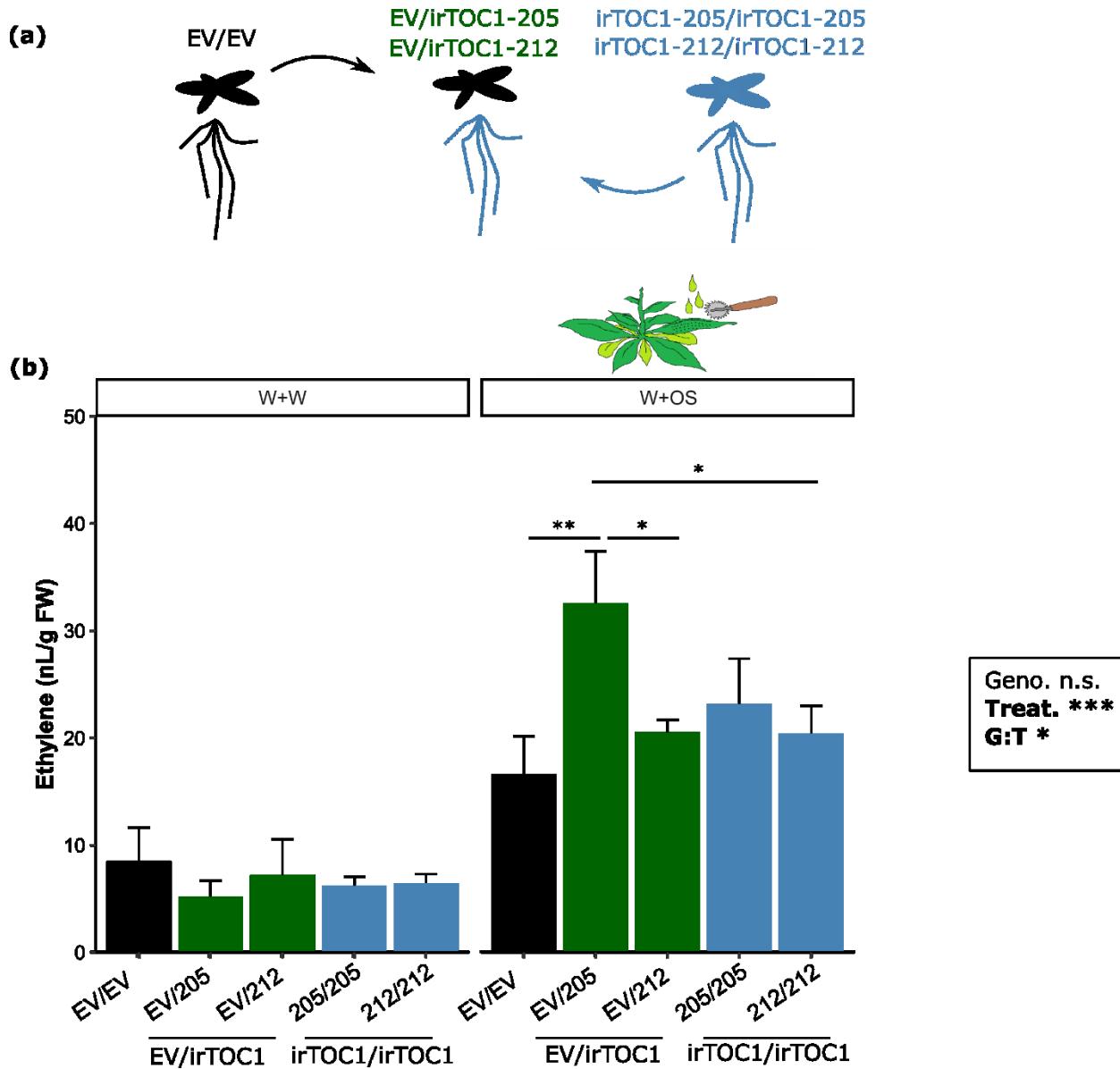


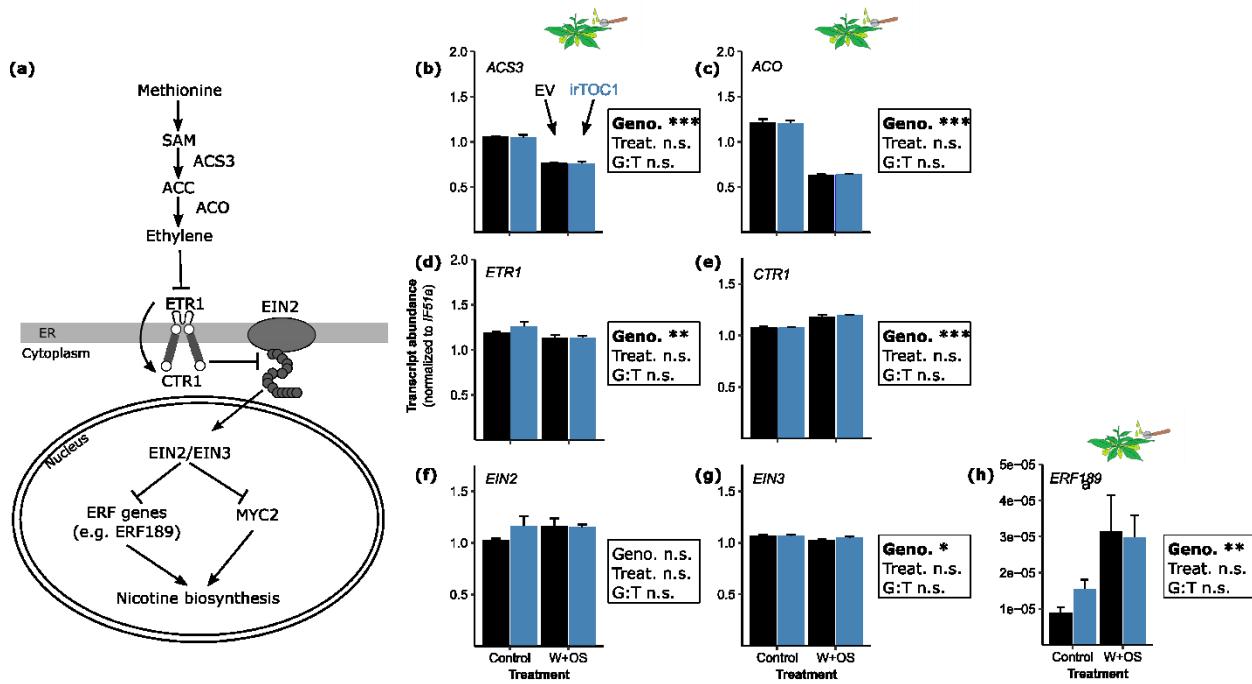
Figure S1

Figure S1: Effect of TOC1 silencing on whole-plant nicotine and phenolamide pools. (a) Experimental design of ^{15}N -labeling experiments: plants were irrigated with ^{15}N -labeled KNO_3 3 days before the start of the experiment, at which plants were treated by controlled wounding and *Manduca sexta* oral secretions (W+OS) and sampled for 93 h to determine shifts in ^{15}N allocation between nicotine and the phenolamides caffeoylputrescine (CP) and dicaffeoyl spermidine (DCS). Effect of silencing irTOC1 on whole-leaf pools of (b) nicotine, (c) CP, and (d) DCS. Effect of silencing TOC1 on whole-leaf pools of ^{15}N -labeled (e) nicotine, (f) CP, and (g) DCS. (N=5 per genotype, treatment and time point). Asterisks represent significant differences in estimated marginal means of linear trends, extracted through Tukey-adjusted pairwise contrasts, within one timepoint: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



20
21 **Figure S2**
22

23 **Figure S2: Ethylene burst after elicitation with *Manduca sexta* oral secretions is not affected by whole-
24 plant nor root-only *TOC1* silencing.** (a) Schematic of two independent silenced lines used for grafting
25 and ethylene analysis in the glasshouse: EV/EV (black), EV/irTOC1 (green), and irTOC1/irTOC1 (blue)
26 micrografted plants. (b) Ethylene levels 5h after elicitation in micrografted EV and *TOC1*-silenced lines.
27 N=6, error bars represent mean + SEM for individual compounds. Asterisks represent significant
differences in estimated marginal means of linear trends, extracted through Tukey-adjusted pairwise
contrasts, within one timepoint: *p<0.05, **p<0.01, ***p<0.001.



28

Figure S3

29 **Figure S3: Effect of *TOC1* silencing on transcript abundance of various ethylene receptors and**
30 **downstream targets 1 hour after simulated herbivory.** (a) Schematic of ethylene biosynthesis and
31 signaling. Ethylene biosynthesis -(b,c), perception (d,e) and downstream signaling (f-g) are significantly
32 affected by W+OS treatment, but not by TOC1 silencing 1h after elicitation. (h) Nicotine biosynthesis-
33 targeting transcription factors, such as ERF189, are not abundant and affected by treatment but not
34 genotype in leaf tissues. N=6, error bars represent mean + SEM for individual compounds. Asterisks
35 represent significant differences in estimated marginal means of linear trends, extracted through Tukey-
36 adjusted pairwise contrasts, within one timepoint: *p<0.05, **p<0.01, ***p<0.001. SAM: S-adenosyl-L-
37 methionine; ACC: 1-Aminocyclopropane-1-carboxylic acid; ACS3: ACC SYNTHASE3; ACO: ACC OXIDASE;
38 ETR1: ETHYLENE RESPONSE1; CTR1: CONSTITUTIVE RESPONSE1; EIN2: ETHYLENE INSENSITIVE2; EIN3:
39 ETHYLENE INSENSITIVE3; ERF189: ETHYLENE RESPONSE FACTOR189

40

(a)



(b)

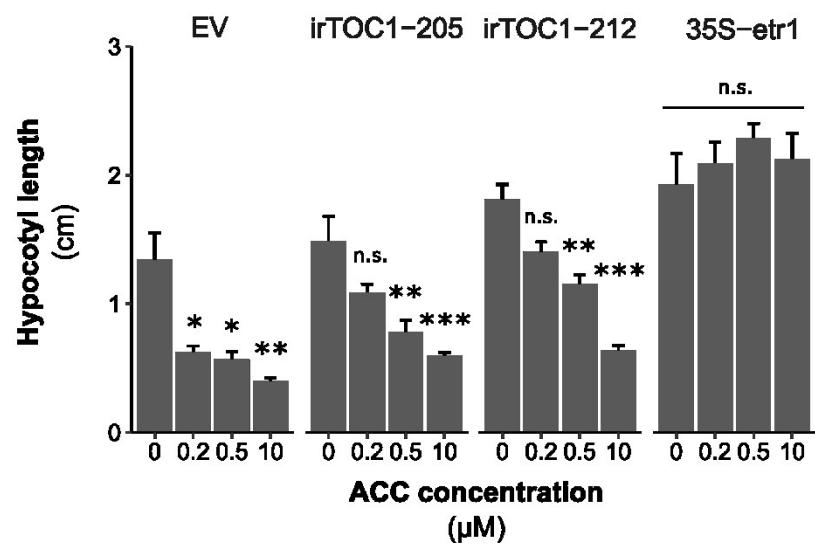
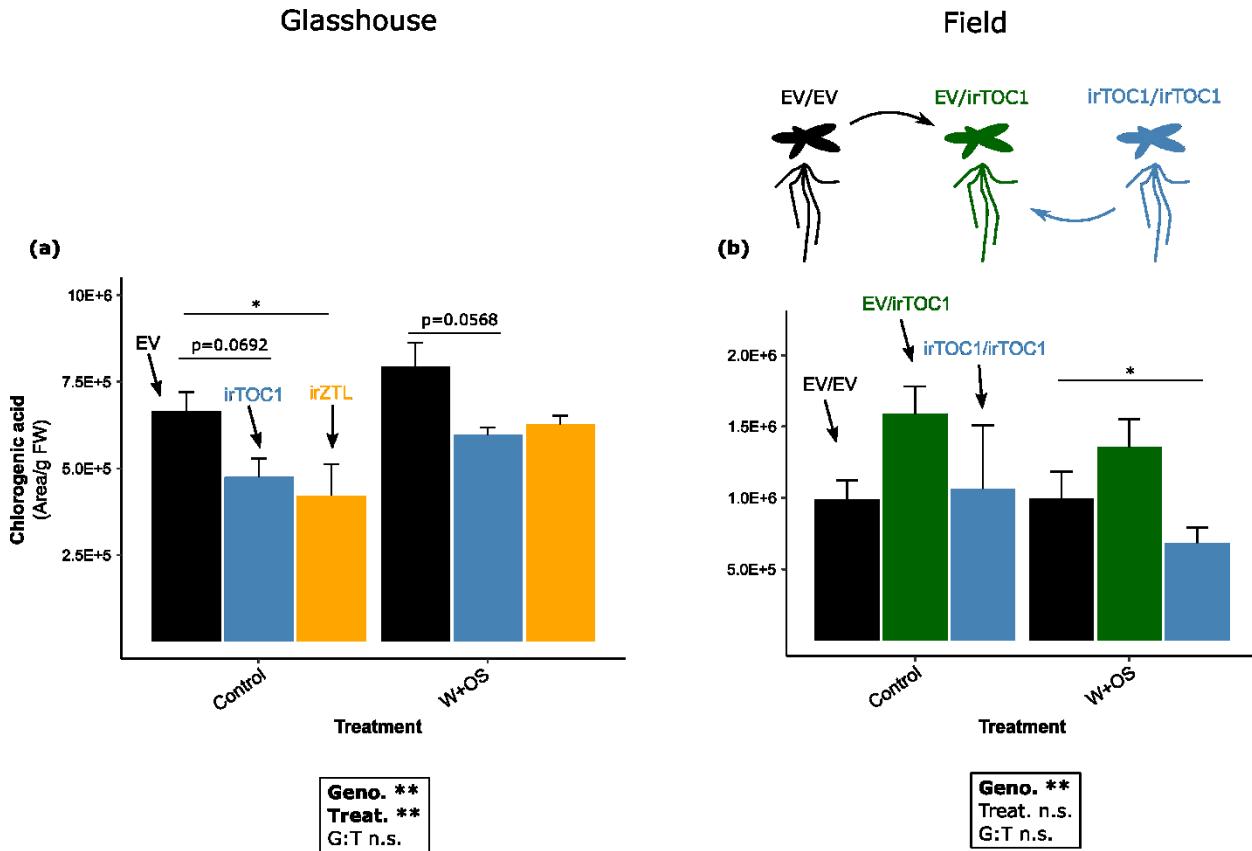


Figure S4

41
42

43 **Figure S4: Effect of silencing *NaTOC1* on ethylene triple response.** (a) Representative seedlings after 10
44 days under dark conditions exposed to ACC concentrations of 0, 0.2, 0.5, and 10 μ M. (b) Hypocotyl
45 lengths of EV, the ethylene-insensitive 35S-etr1 line, and two independent irTOC1 lines. N=4-7 for EV per
46 treatment, 8-10 for other genotypes per treatment, error bars represent mean + SEM for individual
47 compounds. Asterisks represent significant differences in estimated marginal means of linear trends,
48 extracted through Tukey-adjusted pairwise contrasts, within one timepoint: * $p<0.05$, ** $p<0.01$,
49 *** $p<0.001$, ns: not significant.
50



51 **Figure S5: Chlorogenic acid accumulation is attenuated in irTOC1 plants 72h after simulated herbivory.**

52 (a) Levels of chlorogenic acid in EV, irTOC1, and irZTL plants under glasshouse conditions. (b) Schematic
53 of two independent silenced lines used for grafting and ethylene analysis in the glasshouse: EV/EV
54 (black), EV/irTOC1 (green), and irTOC1/irTOC1 (blue) micrografted plants, and levels of chlorogenic acid
55 under field conditions in whole-plant and root-only *TOC1*-silenced plants. N=6 for (a), 4-6 for (b); error
56 bars represent mean + SEM for individual compounds. Asterisks represent significant differences in
57 estimated marginal means of linear trends, extracted through Tukey-adjusted pairwise contrasts, within
58 one timepoint: *p<0.05, **p<0.01, ***p<0.001.
59

60

61 **Table S1: Primer sequences used in this study.**

Gene	Forward	Reverse
IF5a-2 (NIATv7_g63799)	5'-GTCGGACGAAGAACACCATT	5'-CACATCACAGTTGGGAGG
EF1a (NIATv7_g65928)	5'-CCACACTCCCACATTGCTGTCA	5'-CGCATGTCCTCACAGCAAAAC
NaLOX3 (NIATv7_g32174)	5'-GAATTGAGAACAAAGGAGAGTACTG	5'- CTTAACAAATTGTTCCCTCAGATCTCC
NaAOC (NIATv7_g34489)	5'- AACTACCTAACCCCTCTCATTCTCA	5'- AAGCGAAGATAGGCAGGGC
NaOPR3 (NIATv7_g36098)	5'- AATGGAGTTGGAGTTGTTT	5'- AGGTGGTTGAAGCAGTCGTT
NaTD (NIATv7_g00426)	5'-TAAGGCATTGATGGGAGGC	5'-TCTCCCTGTTCACGATAATGGAA
NaMYC2 (NIATv7_g16429)	5'- GGCCCCGAACAACTACTACA	5'- CCCCGTCGATTAAAGTCTGA
NaCOI1 (NIATv7_g03444)	5'- CAGGGCATCTCAGCTGGTC	5'- CGGGATGCTCAGCAACGA
NaMYB8 (NIATv7_g41919)	5'-TCCAAGTAAAAAGCAAAGTCAAG	5'-AGCTTCCCGTGCTCTCAATT
NaACS3 (NIATv7_g57945)	5'- ATTCTCCTTGGTCAGGA	5'- ACTCCAAACTTACTTGTTATTT
NaACO (NIATv7_g18038)	5'- CTATTGAATCTGATGTCAAGCTG	5'- TATGTAGTAGGGACACACGCTT
NaETR1 (NIATv7_g40548)	5'- TGACTGCGCTGGTATCATGTA	5'- TTTCACGATCAAGCTGTGCA
NaCTR1 (NIATv7_g31566)	5'-TATGCAAATCAAGATTGTTGCCA	5'-TCCAAATCAGAAATCCTGTCAGT
NaEIN2 (NIATv7_g03769)	5'-ACTGGCCTTTATTGCTGACA	5'-AGTGCTGTAATCTGATTGGAA
NaEIN3 (NIATv7_g04236)	5'-GGAGGAGGAAGAGATAACCCTCA	5'-TGCAGCCAACAAAGAGACTTA
NaERF189 (NIATv7_g20472)	5'-TACTCCGAGTCCGAGGAAAA	5'-GAAAGCGGCTTGATCGTAAG

62

63

64

65 **Notes S1: Expanded statistical information and outlier removal.**

66 Figure 1:

67 Outlier removal:

68 No outliers were removed from this dataset.

69

70 Transcript abundances:

71 **AOC:**

72 ANOVA results for transcript abundance ("expr") as response variable:

73 Response: expr

74 Df Sum Sq Mean Sq F value Pr(>F)
75 Time1 5 21.0451 4.2090 66.7750 < 2.2e-16 ***
76 Genotype 2 0.0458 0.0229 0.3634 0.697924
77 Time1:Genotype 10 2.0892 0.2089 3.3145 0.004063 **
78 Residuals 35 2.2061 0.0630

79 ---

80 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

81 -----

82 *EMMeans* pairwise contrasts by time point ("Time1"):

83 contrast Time1 estimate SE df t.ratio p.value
84 EV - irTOC1 0 0.07422 0.205 35 0.362 0.9304
85 EV - irZTL 0 0.06926 0.205 35 0.338 0.9391
86 irTOC1 - irZTL 0 -0.00497 0.205 35 -0.024 0.9997
87 EV - irTOC1 30 0.36094 0.205 35 1.761 0.1976
88 EV - irZTL 30 -0.44740 0.205 35 -2.183 0.0883 .
89 irTOC1 - irZTL 30 -0.80835 0.205 35 -3.943 0.0010 **
90 EV - irTOC1 60 -0.12410 0.229 35 -0.541 0.8515
91 EV - irZTL 60 -0.36928 0.229 35 -1.611 0.2544
92 irTOC1 - irZTL 60 -0.24518 0.205 35 -1.196 0.4634
93 EV - irTOC1 90 -0.12317 0.205 35 -0.601 0.8206
94 EV - irZTL 90 -0.25823 0.205 35 -1.260 0.4270
95 irTOC1 - irZTL 90 -0.13506 0.205 35 -0.659 0.7886
96 EV - irTOC1 120 -0.19916 0.205 35 -0.972 0.5994
97 EV - irZTL 120 0.29654 0.205 35 1.447 0.3287
98 irTOC1 - irZTL 120 0.49570 0.205 35 2.418 0.0533 .
99 EV - irTOC1 180 -0.33021 0.205 35 -1.611 0.2545
100 EV - irZTL 180 0.23639 0.205 35 1.153 0.4886
101 irTOC1 - irZTL 180 0.56661 0.205 35 2.764 0.0240 *

102

103 P value adjustment: tukey method for comparing a family of 3 estimates

104 -----

105 *EM Trends* pairwise contrasts with transcript abundance against Time
106 ("Time"):

107 \$emtrends

108 Genotype Time.trend SE df lower.CL upper.CL
109 EV 0.00863 0.00184 47 0.00492 0.01233

```

110    irTOC1      0.01164 0.00183 47  0.00795  0.01533
111    irZTL       0.00589 0.00183 47  0.00220  0.00958
112
113 Confidence level used: 0.95
114
115 $contrasts
116   contrast      estimate      SE df t.ratio p.value
117   EV - irTOC1   -0.00302 0.00260 47 -1.161  0.4821
118   EV - irZTL    0.00274 0.00260 47  1.054  0.5470
119   irTOC1 - irZTL 0.00576 0.00259 47  2.219  0.0784 .
120
121 P value adjustment: tukey method for comparing a family of 3 estimates
122
123 COI1:
124 ANOVA results for transcript abundance ("expr") as response variable:
125 Response: expr
126
127   Df Sum Sq Mean Sq F value    Pr(>F)
128 Time1      5 37.759 7.5518 51.6868 1.167e-14 ***
129 Genotype     2  0.162  0.0808  0.5527  0.58063
130 Time1:Genotype 10  4.255  0.4255  2.9121  0.01002 *
131 Residuals    33  4.822  0.1461
132
133 Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
134
135 EMMeans pairwise contrasts by time point ("Time1"):
136 contrast      Time1 estimate      SE df t.ratio p.value
137   EV - irTOC1    0     0.50865 0.312 33  1.630  0.2476
138   EV - irZTL     0     1.33528 0.441 33  3.025  0.0129 *
139   irTOC1 - irZTL 0     0.82663 0.441 33  1.873  0.1625
140   EV - irTOC1   30    -0.79094 0.312 33 -2.534  0.0418 *
141   EV - irZTL   30     0.43901 0.312 33  1.407  0.3492
142   irTOC1 - irZTL 30    1.22995 0.312 33  3.941  0.0011 **
143   EV - irTOC1   60     0.39290 0.349 33  1.126  0.5052
144   EV - irZTL   60     0.04688 0.349 33  0.134  0.9901
145   irTOC1 - irZTL 60    -0.34603 0.312 33 -1.109  0.5156
146   EV - irTOC1   90     0.28191 0.312 33  0.903  0.6421
147   EV - irZTL   90     0.03168 0.312 33  0.101  0.9943
148   irTOC1 - irZTL 90    -0.25023 0.312 33 -0.802  0.7046
149   EV - irTOC1  120     0.00236 0.312 33  0.008  1.0000
150   EV - irZTL  120    -0.10542 0.312 33 -0.338  0.9392
151   irTOC1 - irZTL 120   -0.10777 0.312 33 -0.345  0.9365
152   EV - irTOC1  180    -0.32190 0.312 33 -1.031  0.5627
153   EV - irZTL  180    -0.40807 0.312 33 -1.308  0.4011
154   irTOC1 - irZTL 180   -0.08617 0.312 33 -0.276  0.9589
155
156 P value adjustment: tukey method for comparing a family of 3 estimates

```

```

157 EM Trends pairwise contrasts with transcript abundance against Time
158 ("Time"):
159 $emtrends
160   Genotype Time.trend      SE df lower.CL upper.CL
161   EV        -0.0147 0.00271 45  -0.0202 -0.009257
162   irTOC1    -0.0127 0.00270 45  -0.0182 -0.007302
163   irZTL     -0.0053 0.00308 45  -0.0115  0.000904
164
165 Confidence level used: 0.95
166
167 $contrasts
168   contrast   estimate      SE df t.ratio p.value
169   EV - irTOC1 -0.00197 0.00383 45 -0.515  0.8645
170   EV - irZTL  -0.00942 0.00410 45 -2.297  0.0665 .
171   irTOC1 - irZTL -0.00745 0.00410 45 -1.819  0.1751
172
173 P value adjustment: tukey method for comparing a family of 3 estimates
174
175 LOX3:
176 ANOVA results for transcript abundance ("expr") as response variable:
177 Response: expr
178           Df Sum Sq Mean Sq F value    Pr(>F)
179 Time1       5 31.2535 6.2507 101.015 < 2.2e-16 ***
180 Genotype     2  3.5390 1.7695  28.596 3.670e-08 ***
181 Time1:Genotype 10  6.5647 0.6565  10.609 4.524e-08 ***
182 Residuals    36  2.2276 0.0619
183 ---
184 Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
185 -----
186 EMMMeans pairwise contrasts by time point ("Time1"):
187 contrast   Time1 estimate      SE df t.ratio p.value
188 EV - irTOC1  0     0.03491 0.203 36  0.172  0.9839
189 EV - irZTL   0     0.04726 0.203 36  0.233  0.9706
190 irTOC1 - irZTL 0     0.01235 0.203 36  0.061  0.9980
191 EV - irTOC1  30    0.71325 0.203 36  3.512  0.0034 **
192 EV - irZTL   30    -1.24614 0.203 36 -6.135 <.0001 ***
193 irTOC1 - irZTL 30    -1.95938 0.203 36 -9.647 <.0001 ***
194 EV - irTOC1  60    -0.78493 0.203 36 -3.865  0.0013 **
195 EV - irZTL   60    -0.66602 0.203 36 -3.279  0.0064 **
196 irTOC1 - irZTL 60    0.11892 0.203 36  0.585  0.8287
197 EV - irTOC1  90    -0.70667 0.203 36 -3.479  0.0037 **
198 EV - irZTL   90    -1.40826 0.203 36 -6.934 <.0001 ***
199 irTOC1 - irZTL 90    -0.70158 0.203 36 -3.454  0.0040 **
200 EV - irTOC1 120    0.00818 0.203 36  0.040  0.9991
201 EV - irZTL 120    -0.25737 0.203 36 -1.267  0.4226
202 irTOC1 - irZTL 120   -0.26555 0.203 36 -1.307  0.4003
203 EV - irTOC1 180    -0.09430 0.203 36 -0.464  0.8883
204 EV - irZTL 180    -0.06243 0.203 36 -0.307  0.9493

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205  irTOC1 - irZTL 180      0.03187 0.203 36  0.157  0.9865
206
207 P value adjustment: tukey method for comparing a family of 3 estimates
208 -----
209 EM Trends pairwise contrasts with transcript abundance against Time
210 ("Time"):
211 $emtrends
212   Genotype Time.trend      SE df lower.CL upper.CL
213   EV        -0.000717 0.00361 48 -0.00798  0.00655
214   irTOC1    0.001137 0.00361 48 -0.00613  0.00840
215   irZTL     -0.002680 0.00361 48 -0.00994  0.00458
216
217 Confidence level used: 0.95
218
219 $contrasts
220   contrast   estimate      SE df t.ratio p.value
221   EV - irTOC1 -0.00185 0.00511 48 -0.363  0.9301
222   EV - irZTL  0.00196 0.00511 48  0.384  0.9220
223   irTOC1 - irZTL 0.00382 0.00511 48  0.747  0.7369
224
225 P value adjustment: tukey method for comparing a family of 3 estimates
226
227 MYB8:
228 ANOVA results for transcript abundance ("expr") as response variable:
229 Response: expr
230             Df Sum Sq Mean Sq F value    Pr(>F)
231 Time1          5 60.234 12.0467 477.804 < 2.2e-16 ***
232 Genotype       2  5.908  2.9540 117.163 < 2.2e-16 ***
233 Time1:Genotype 10 21.935  2.1935  86.998 < 2.2e-16 ***
234 Residuals      36  0.908  0.0252
235 ---
236 Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
237 -----
238 EMMeans pairwise contrasts by time point ("Time1"):
239 contrast   Time1 estimate      SE df t.ratio p.value
240   EV - irTOC1    0     0.1384 0.13 36  1.067  0.5402
241   EV - irZTL     0     0.0955 0.13 36  0.737  0.7435
242   irTOC1 - irZTL 0    -0.0429 0.13 36 -0.331  0.9416
243   EV - irTOC1   30    1.6501 0.13 36 12.728 <.0001 ***
244   EV - irZTL    30   -0.8798 0.13 36 -6.786 <.0001 ***
245   irTOC1 - irZTL 30   -2.5299 0.13 36 -19.514 <.0001 ***
246   EV - irTOC1   60    2.9016 0.13 36 22.381 <.0001 ***
247   EV - irZTL    60    2.9134 0.13 36 22.472 <.0001 ***
248   irTOC1 - irZTL 60    0.0118 0.13 36  0.091  0.9955
249   EV - irTOC1   90    0.6163 0.13 36  4.754  0.0001 ***
250   EV - irZTL    90    0.4326 0.13 36  3.337  0.0055 **
251   irTOC1 - irZTL 90   -0.1837 0.13 36 -1.417  0.3430
252   EV - irTOC1  120   -0.2173 0.13 36 -1.676  0.2280

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253   EV - irZTL    120      0.2356 0.13 36    1.817 0.1785
254   irTOC1 - irZTL 120      0.4529 0.13 36    3.493 0.0036 ** 
255   EV - irTOC1    180     -0.2425 0.13 36   -1.870 0.1620
256   EV - irZTL    180     -0.0475 0.13 36   -0.366 0.9289
257   irTOC1 - irZTL 180      0.1950 0.13 36    1.504 0.3010
258
259 P value adjustment: tukey method for comparing a family of 3 estimates
260 -----
261 EM Trends pairwise contrasts with transcript abundance against Time
262 ("Time"):
263 $mtrends
264   Genotype Time.trend      SE df lower.CL upper.CL
265   EV        -0.00674 0.00507 48 -0.01693 0.00345
266   irTOC1     0.00175 0.00507 48 -0.00844 0.01194
267   irZTL     -0.00613 0.00507 48 -0.01632 0.00406
268
269 Confidence level used: 0.95
270
271 $contrasts
272   contrast      estimate      SE df t.ratio p.value
273   EV - irTOC1   -0.008495 0.00717 48 -1.185 0.4676
274   EV - irZTL    -0.000615 0.00717 48 -0.086 0.9959
275   irTOC1 - irZTL 0.007879 0.00717 48  1.099 0.5191
276
277 P value adjustment: tukey method for comparing a family of 3 estimates
278
279 MYC2:
280 ANOVA results for transcript abundance ("expr") as response variable:
281 Response: expr
282   Df Sum Sq Mean Sq F value    Pr(>F)
283 Time1          5 33.608  6.7217 164.557 < 2.2e-16 ***
284 Genotype       2  2.272  1.1362  27.816 5.872e-08 ***
285 Time1:Genotype 10  5.995  0.5995  14.677 8.950e-10 ***
286 Residuals      35  1.430  0.0408
287 ---
288 Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
289 -----
290 EMMeans pairwise contrasts by time point ("Time1"):
291 contrast      Time1 estimate      SE df t.ratio p.value
292   EV - irTOC1    0      -0.0353 0.165 35 -0.214 0.9751
293   EV - irZTL     0      -0.0712 0.165 35 -0.432 0.9027
294   irTOC1 - irZTL 0      -0.0360 0.165 35 -0.218 0.9742
295   EV - irTOC1   30      2.0008 0.165 35 12.125 <.0001 ***
296   EV - irZTL    30      1.8269 0.165 35 11.071 <.0001 ***
297   irTOC1 - irZTL 30     -0.1739 0.165 35 -1.054 0.5487
298   EV - irTOC1   60      0.7174 0.184 35  3.888 0.0012 **
299   EV - irZTL    60      0.5789 0.165 35  3.508 0.0035 **
300   irTOC1 - irZTL 60     -0.1385 0.184 35 -0.751 0.7352

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301   EV - irTOC1    90      0.1437 0.165 35  0.871  0.6621
302   EV - irZTL     90      0.0136 0.165 35  0.082  0.9963
303   irTOC1 - irZTL 90     -0.1301 0.165 35 -0.788  0.7126
304   EV - irTOC1    120     -0.0265 0.165 35 -0.160  0.9859
305   EV - irZTL     120      0.0766 0.165 35  0.464  0.8884
306   irTOC1 - irZTL 120      0.1030 0.165 35  0.624  0.8078
307   EV - irTOC1    180     -0.0592 0.165 35 -0.359  0.9317
308   EV - irZTL     180      0.1308 0.165 35  0.792  0.7101
309   irTOC1 - irZTL 180      0.1899 0.165 35  1.151  0.4898
310
311 P value adjustment: tukey method for comparing a family of 3 estimates
312 -----
313 EM Trends pairwise contrasts with transcript abundance against Time
314 ("Time"):
315 $emtrends
316 Genotype Time.trend      SE df lower.CL upper.CL
317 EV         -0.00847 0.00341 47 -0.01532 -0.00162
318 irTOC1     -0.00259 0.00342 47 -0.00947  0.00428
319 irZTL      -0.00461 0.00341 47 -0.01147  0.00224
320
321 Confidence level used: 0.95
322
323 $contrasts
324 contrast      estimate      SE df t.ratio p.value
325 EV - irTOC1   -0.00587 0.00483 47 -1.217  0.4491
326 EV - irZTL    -0.00385 0.00482 47 -0.800  0.7048
327 irTOC1 - irZTL 0.00202 0.00483 47  0.418  0.9082
328
329 P value adjustment: tukey method for comparing a family of 3 estimates
330
331 OPR3:
332 ANOVA results for transcript abundance ("expr") as response variable:
333 Response: expr
334           Df Sum Sq Mean Sq F value    Pr(>F)
335 Time1        5 28.2907  5.6581 124.6630 < 2.2e-16 ***
336 Genotype     2  0.5407  0.2703   5.9564 0.0058251  **
337 Time1:Genotype 10  2.2809  0.2281   5.0254 0.0001454 ***
338 Residuals    36  1.6340  0.0454
339 ---
340 Signif. codes:  0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
341 -----
342 EMMMeans pairwise contrasts by time point ("Time1"):
343 contrast      Time1 estimate      SE df t.ratio p.value
344 EV - irTOC1    0      0.06775 0.174 36  0.389  0.9200
345 EV - irZTL     0      0.06303 0.174 36  0.362  0.9303
346 irTOC1 - irZTL 0     -0.00472 0.174 36 -0.027  0.9996
347 EV - irTOC1    30     0.39011 0.174 36  2.243  0.0775
348 EV - irZTL    30     -0.86445 0.174 36 -4.970 <.0001 ***

```

```

349  irTOC1 - irZTL 30      -1.25457 0.174 36 -7.212 <.0001 ***
350  EV - irTOC1       60      -0.02744 0.174 36 -0.158  0.9864
351  EV - irZTL        60      -0.05000 0.174 36 -0.287  0.9555
352  irTOC1 - irZTL 60      -0.02256 0.174 36 -0.130  0.9908
353  EV - irTOC1       90      0.07395 0.174 36  0.425  0.9054
354  EV - irZTL        90      -0.19938 0.174 36 -1.146  0.4925
355  irTOC1 - irZTL 90      -0.27333 0.174 36 -1.571  0.2709
356  EV - irTOC1      120      0.32732 0.174 36  1.882  0.1586
357  EV - irZTL       120      0.25149 0.174 36  1.446  0.3288
358  irTOC1 - irZTL 120     -0.07583 0.174 36 -0.436  0.9008
359  EV - irTOC1      180     -0.05917 0.174 36 -0.340  0.9383
360  EV - irZTL       180      0.10184 0.174 36  0.585  0.8287
361  irTOC1 - irZTL 180     0.16100 0.174 36  0.926  0.6279
362
363 P value adjustment: tukey method for comparing a family of 3 estimates
364 -----
365 EM Trends pairwise contrasts with transcript abundance against Time
366 ("Time"):
367 $emtrends
368   Genotype Time.trend      SE df lower.CL upper.CL
369   EV          0.00417 0.00312 48 -0.00211   0.0104
370   irTOC1      0.00495 0.00312 48 -0.00132   0.0112
371   irZTL       0.00143 0.00312 48 -0.00484   0.0077
372
373 Confidence level used: 0.95
374
375 $contrasts
376   contrast      estimate      SE df t.ratio p.value
377   EV - irTOC1    -0.000784 0.00441 48 -0.178  0.9828
378   EV - irZTL     0.002735 0.00441 48  0.620  0.8101
379   irTOC1 - irZTL 0.003519 0.00441 48  0.797  0.7064
380
381 P value adjustment: tukey method for comparing a family of 3 estimates
382
383 TD:
384 ANOVA results for transcript abundance ("expr") as response variable:
385 Response: expr
386             Df Sum Sq Mean Sq F value    Pr(>F)
387 Time1          5 57.587 11.5173 13.1773 9.732e-07 ***
388 Genotype       2  0.703  0.3513  0.4019   0.67270
389 Time1:Genotype 10 21.571  2.1571  2.4680   0.02815 *
390 Residuals      29 25.347  0.8740
391 ---
392 Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
393 -----
394 EMeans pairwise contrasts by time point ("Time1"):
395 contrast      Time1 estimate      SE df t.ratio p.value
396 EV - irTOC1    0      0.2997 0.763 29  0.393  0.9188

```

```

497   EV - irZTL      0      0.2087 1.080 29  0.193  0.9796
498   irTOC1 - irZTL 0     -0.0910 1.080 29 -0.084  0.9961
499   EV - irTOC1    30     0.1278 0.763 29  0.167  0.9847
500   EV - irZTL    30     0.0236 0.763 29  0.031  0.9995
501   irTOC1 - irZTL 30    -0.1041 0.763 29 -0.136  0.9898
502   EV - irTOC1    60     0.1333 1.080 29  0.123  0.9916
503   EV - irZTL    60     0.1803 1.145 29  0.157  0.9864
504   irTOC1 - irZTL 60     0.0470 0.853 29  0.055  0.9983
505   EV - irTOC1    90     0.6131 0.853 29  0.718  0.7547
506   EV - irZTL    90     -1.1260 0.853 29 -1.319  0.3961
507   irTOC1 - irZTL 90     -1.7392 0.763 29 -2.278  0.0750 .
508   EV - irTOC1   120     -0.6416 0.763 29 -0.841  0.6812
509   EV - irZTL   120     -0.3194 0.763 29 -0.418  0.9083
510   irTOC1 - irZTL 120     0.3223 0.763 29  0.422  0.9067
511   EV - irTOC1   180     -1.8818 0.763 29 -2.465  0.0505 .
512   EV - irZTL   180     1.8306 0.853 29  2.145  0.0983 .
513   irTOC1 - irZTL 180     3.7124 0.853 29  4.350  0.0004 ***
514
515 P value adjustment: tukey method for comparing a family of 3 estimates
516 -----
517 EM Trends pairwise contrasts with transcript abundance against Time
518 ("Time"):
519 $emtrends
520   Genotype Time.trend      SE df lower.CL upper.CL
521   EV          0.01504 0.00408 41  0.006805  0.0233
522   irTOC1      0.02672 0.00405 41  0.018544  0.0349
523   irZTL       0.00948 0.00512 41 -0.000862  0.0198
524
525 Confidence level used: 0.95
526
527 $contrasts
528   contrast      estimate      SE df t.ratio p.value
529   EV - irTOC1   -0.01168 0.00575 41 -2.032  0.1173
530   EV - irZTL    0.00556 0.00655 41  0.850  0.6747
531   irTOC1 - irZTL 0.01724 0.00653 41  2.641  0.0306 *
532
533 P value adjustment: tukey method for comparing a family of 3 estimates
534
535 Phytohormones:
536 JA:
537 ANOVA results for ng/g fresh weight (FW) ("Con") as response variable:
538 Response: Con
539             Df Sum Sq Mean Sq F value    Pr(>F)
540 Time1          5 24367477 4873495  52.432 < 2.2e-16 ***
541 Genotype        2 1609492  804746   8.658 0.0003639 ***
542 Time1:Genotype 10 3185340  318534   3.427 0.0007707 ***
543 Residuals       90 8365353  92948

```

```

444 ---
445 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
446 -----
447 EMMeans pairwise contrasts by time point ("Time1"):
448 contrast Time1 estimate SE df t.ratio p.value
449 EV - irZTL 0 27.34 176 90 0.155 0.9868
450 EV - TOC 0 18.43 176 90 0.105 0.9940
451 irZTL - TOC 0 -8.92 176 90 -0.051 0.9986
452 EV - irZTL 30 -19.79 176 90 -0.112 0.9931
453 EV - TOC 30 219.27 176 90 1.246 0.4296
454 irZTL - TOC 30 239.06 176 90 1.358 0.3672
455 EV - irZTL 60 922.94 176 90 5.243 <.0001 ***
456 EV - TOC 60 344.85 176 90 1.959 0.1284
457 irZTL - TOC 60 -578.09 176 90 -3.284 0.0041 **
458 EV - irZTL 90 758.68 176 90 4.310 0.0001 ***
459 EV - TOC 90 214.49 176 90 1.219 0.4454
460 irZTL - TOC 90 -544.19 176 90 -3.092 0.0074 **
461 EV - irZTL 120 141.95 176 90 0.806 0.7000
462 EV - TOC 120 -33.78 176 90 -0.192 0.9799
463 irZTL - TOC 120 -175.73 176 90 -0.998 0.5798
464 EV - irZTL 180 -56.17 176 90 -0.319 0.9455
465 EV - TOC 180 -102.47 176 90 -0.582 0.8300
466 irZTL - TOC 180 -46.31 176 90 -0.263 0.9626
467
468 P value adjustment: tukey method for comparing a family of 3 estimates
469 -----
470 EM Trends pairwise contrasts with JA concentration against Time
471 ("Time"):
472 $emtrends
473 Genotype Time.trend SE df lower.CL upper.CL
474 EV -0.6577 1.67 102 -3.97 2.65
475 irZTL -0.0858 1.67 102 -3.40 3.22
476 TOC 0.7132 1.67 102 -2.60 4.02
477
478 Confidence level used: 0.95
479
480 $contrasts
481 contrast estimate SE df t.ratio p.value
482 EV - irZTL -0.572 2.36 102 -0.242 0.9682
483 EV - TOC -1.371 2.36 102 -0.581 0.8307
484 irZTL - TOC -0.799 2.36 102 -0.339 0.9388
485
486 P value adjustment: tukey method for comparing a family of 3 estimates
487
488 JA-Ile:
489 ANOVA results for concentration, ng/g FW ("Con") as response variable:
490 Response: Con
491 Df Sum Sq Mean Sq F value Pr(>F)

```

```
492 Time1      5 344078    68816   76.029 < 2.2e-16 ***
493 Genotype    2 40692     20346   22.479 1.208e-08 ***
494 Time1:Genotype 10 136584    13658   15.090 1.970e-15 ***
495 Residuals   90 81461      905
496 ---
497 Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
498 -----
499 EMMMeans pairwise contrasts by time point ("Time1"):
```

```

500 contrast      Time1 estimate    SE df t.ratio p.value
501   EV - irZTL  0       1.287 17.4 90  0.074  0.9970
502   EV - TOC    0       1.866 17.4 90  0.107  0.9937
503   irZTL - TOC 0       0.579 17.4 90  0.033  0.9994
504   EV - irZTL 30      26.551 17.4 90  1.529  0.2825
505   EV - TOC   30      63.436 17.4 90  3.652  0.0013 ** 
506   irZTL - TOC 30     36.885 17.4 90  2.124  0.0908 .
507   EV - irZTL 60      53.726 17.4 90  3.093  0.0074 ** 
508   EV - TOC   60      -81.621 17.4 90 -4.699 <.0001 *** 
509   irZTL - TOC 60     -135.346 17.4 90 -7.792 <.0001 *** 
510   EV - irZTL 90      181.194 17.4 90 10.432 <.0001 *** 
511   EV - TOC   90      139.473 17.4 90  8.030 <.0001 *** 
512   irZTL - TOC 90     -41.722 17.4 90 -2.402  0.0478 * 
513   EV - irZTL 120     20.642 17.4 90  1.188  0.4631
514   EV - TOC   120     9.062 17.4 90  0.522  0.8609
515   irZTL - TOC 120    -11.580 17.4 90 -0.667  0.7834
516   EV - irZTL 180     1.589 17.4 90  0.091  0.9954
517   EV - TOC   180     -0.869 17.4 90 -0.050  0.9986
518   irZTL - TOC 180    -2.458 17.4 90 -0.142  0.9890
519
520 P value adjustment: tukey method for comparing a family of 3 estimates
521 -----
522 EM Trends pairwise contrasts with JA-Ile concentration against Time
523 ("Time"):
524 $emtrends
525 Genotype Time.trend    SE  df lower.CL upper.CL
526   EV       -0.0164 0.209 102   -0.431   0.398
527   irZTL    -0.0303 0.209 102   -0.445   0.384
528   TOC      -0.0155 0.209 102   -0.430   0.399
529
530 Confidence level used: 0.95
531
532 $contrasts
533 contrast      estimate    SE  df t.ratio p.value
534   EV - irZTL  0.013881 0.296 102  0.047  0.9988
535   EV - TOC    -0.000876 0.296 102 -0.003  1.0000
536   irZTL - TOC -0.014757 0.296 102 -0.050  0.9986
537
538 P value adjustment: tukey method for comparing a family of 3 estimates
539
```

540 Figure 2:

541 Outlier removal:

542 No outliers were removed from this dataset.

543

```

544 Secondary metabolites:
545 Nicotine:
546 ANOVA results for nicotine concentration Peak area/g FW ("Nicotine")
547 as response variable:
548 Response: Nicotine
549                               Df Sum Sq Mean Sq F value    Pr(>F)
550 as.factor(Time)            1 368.53 368.53 20.0430 0.0001015 ***
551 Genotype                  2 577.52 288.76 15.7047 2.154e-05 ***
552 as.factor(Time):Genotype 2 273.66 136.83 7.4416 0.0023744 **
553 Residuals                 30 551.61 18.39
554 ---
555 Signif. codes: 0 '****' 0.001 '***' 0.01 '**' 0.05 '*' 0.1 '.' 1
556 -----
557 EMMMeans pairwise contrasts by time point in hours ("Time"):
558 contrast      Time estimate   SE df t.ratio p.value
559 EV - irTOC1    0     -0.419 2.48 30 -0.169 0.9843
560 EV - irZTL     0      2.786 2.48 30  1.125 0.5062
561 irTOC1 - irZTL 0      3.205 2.48 30  1.295 0.4091
562 EV - irTOC1    72    -9.458 2.48 30 -3.821 0.0018 **
563 EV - irZTL     72     6.958 2.48 30  2.811 0.0228
564 irTOC1 - irZTL 72     16.417 2.48 30  6.631 <.0001 ***
565
566 P value adjustment: tukey method for comparing a family of 3 estimates
567
568 Caffeoylputrescine:
569 ANOVA results for caffeoylputrescine concentration Peak area/g FW
570 ("CP") as response variable:
571 Response: CP
572                               Df Sum Sq Mean Sq F value    Pr(>F)
573 as.factor(Time)            1 66.115 66.115 48.4932 9.786e-08 ***
574 Genotype                  2 13.437 6.719  4.9279  0.01411 *
575 as.factor(Time):Genotype  2  7.559 3.780  2.7723  0.07856 .
576 Residuals                 30 40.902 1.363
577 ---
578 Signif. codes: 0 '****' 0.001 '***' 0.01 '**' 0.05 '*' 0.1 '.' 1
579 -----
580 EMMMeans pairwise contrasts by time point in hours ("Time"):
581 contrast      Time estimate   SE df t.ratio p.value
582 EV - irTOC1    0     0.372 0.674 30  0.551 0.8466
583 EV - irZTL     0     0.352 0.674 30  0.522 0.8614
584 irTOC1 - irZTL 0    -0.020 0.674 30 -0.030 0.9995
585 EV - irTOC1    72    2.612 0.674 30  3.874 0.0015 **
586 EV - irZTL     72    1.346 0.674 30  1.997 0.1304
587 irTOC1 - irZTL 72   -1.266 0.674 30 -1.877 0.1627
588
589 P value adjustment: tukey method for comparing a family of 3 estimates
590

```

```

591 Dicaffeoylspermidine:
592 ANOVA results for dicaffeoylspermidine concentration Peak area/g FW
593 ("DCS") as response variable:
594 Response: DCS
595             Df  Sum Sq Mean Sq F value    Pr(>F)
596 as.factor(Time)      1  0.1148  0.1148  0.2928  0.5924389
597 Genotype            2  7.4137  3.7069  9.4543  0.0006547 *** 
598 as.factor(Time):Genotype 2  0.3819  0.1909  0.4870  0.6192691
599 Residuals          30 11.7625  0.3921
600 ---
601 Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
602 -----
603 EMMeans pairwise contrasts by time point in hours ("Time"):
604 contrast   Time estimate   SE df t.ratio p.value
605 EV - irTOC1 0       1.296 0.362 30  3.585  0.0033 ** 
606 EV - irZTL  0       0.988 0.362 30  2.732  0.0274 *
607 irTOC1 - irZTL 0     -0.309 0.362 30 -0.853  0.6733
608 EV - irTOC1  72     0.865 0.362 30  2.393  0.0585 .
609 EV - irZTL   72     0.545 0.362 30  1.507  0.3020
610 irTOC1 - irZTL 72   -0.320 0.362 30 -0.886  0.6533
611
612 P value adjustment: tukey method for comparing a family of 3 estimates
613
614 Trait correlations:
615 Nicotine vs. caffeoyleputrescine:
616 ANOVA results for nicotine concentration ("Nicotine") as response
617 variable:
618 Response: Nicotine
619             Df  Sum Sq Mean Sq F value    Pr(>F)
620 CP        1 137.65 137.65  8.1299  0.007805 ** 
621 Genotype  2 694.77 347.39 20.5172 2.425e-06 *** 
622 CP:Genotype 2 430.96 215.48 12.7265 9.952e-05 *** 
623 Residuals 30 507.94 16.93
624 ---
625 Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
626 -----
627 EMMeans pairwise contrasts by CP level ("CP"):
628 contrast   CP estimate   SE df t.ratio p.value
629 EV - irTOC1 1.55     -11.79 2.00 30 -5.896 <.0001 ***
630 EV - irZTL  1.55      3.89 1.72 30  2.258  0.0778 .
631 irTOC1 - irZTL 1.55    15.68 1.96 30  7.994 <.0001 ***
632
633 P value adjustment: tukey method for comparing a family of 3 estimates
634 -----
635 EM Trends pairwise contrasts with nicotine against CP concentration
636 ("CP.trend"):
637 $emtrends

```

```

638 Genotype CP.trend SE df lower.CL upper.CL
639 EV 1.211 0.498 30 0.195 2.23
640 irTOC1 8.297 1.422 30 5.393 11.20
641 irZTL 0.492 0.669 30 -0.875 1.86
642
643 Confidence level used: 0.95
644
645 $contrasts
646 contrast estimate SE df t.ratio p.value
647 EV - irTOC1 -7.086 1.506 30 -4.704 0.0002 ***
648 EV - irZTL 0.718 0.834 30 0.861 0.6683
649 irTOC1 - irZTL 7.805 1.572 30 4.966 0.0001 ***
650
651 P value adjustment: tukey method for comparing a family of 3 estimates
652
653 Nicotine vs. dicaffeoylspermidine:
654 ANOVA results nicotine concentration ("Nicotine") as response
655 variable:
656 Response: Nicotine
657 Df Sum Sq Mean Sq F value Pr(>F)
658 DCS 1 13.31 13.305 0.3730 0.545996
659 Genotype 2 564.76 282.379 7.9152 0.001736 **
660 DCS:Genotype 2 122.99 61.494 1.7237 0.195607
661 Residuals 30 1070.27 35.676
662 ---
663 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
664 -----
665 EMMeans pairwise contrasts by DCS level ("DCS"):
666 contrast DCS estimate SE df t.ratio p.value
667 EV - irTOC1 0.87 -9.85 3.98 30 -2.478 0.0486 *
668 EV - irZTL 0.87 5.42 2.81 30 1.931 0.1474
669 irTOC1 - irZTL 0.87 15.27 3.88 30 3.941 0.0013 **
670
671 P value adjustment: tukey method for comparing a family of 3 estimates
672 -----
673 EM Trends pairwise contrasts with nicotine against DCS concentration
674 ("DCS.trend"):
675 $emtrends
676 Genotype DCS.trend SE df lower.CL upper.CL
677 EV -0.631 1.90 30 -4.5 3.24
678 irTOC1 11.405 6.27 30 -1.4 24.21
679 irZTL -1.058 5.00 30 -11.3 9.15
680
681 Confidence level used: 0.95
682
683 $contrasts
684 contrast estimate SE df t.ratio p.value
685 EV - irTOC1 -12.036 6.55 30 -1.837 0.1750

```

```
686    EV - irZTL      0.427 5.35 30  0.080  0.9965
687    irTOC1 - irZTL  12.463 8.02 30  1.554   0.2809
688
689 P value adjustment: tukey method for comparing a family of 3 estimates
690
691
```

692 Figures 3, 4, and S1:
 693 Outlier removal:
 694 One data point (21h control treatment, irTOC1 replicate 3) did not
 695 produce clear peaks for nicotine and CP and was removed before
 696 analysis.
 697 Secondary metabolite concentrations (Figure 3b,c,d):
698 Nicotine:
 699 ANOVA results for nicotine concentration in ug/mL ("Nic_ug_mL") as
 700 response variable:
 701 Response: Nic_ug_mL

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
702 Genotype	1	100135	100135	189.9925	< 2.2e-16 ***
703 Treatment	1	339606	339606	644.3537	< 2.2e-16 ***
704 Timepoint	7	75975	10854	20.5930	< 2.2e-16 ***
705 Genotype:Treatment	1	10308	10308	19.5574	2.272e-05 ***
706 Genotype:Timepoint	7	69089	9870	18.7266	2.408e-16 ***
707 Treatment:Timepoint	5	17581	3516	6.6713	1.786e-05 ***
708 Genotype:Treatment:Timepoint	5	13854	2771	5.2571	0.0002248 ***
709 Residuals	112	59029	527		
710	---				
711 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
712	-----				
713 EMMeans pairwise contrasts by treatment and time point in hours ("Treatment", "Timepoint"):					
714 contrast Treatment Timepoint estimate SE df t.ratio p.value					
715 EV - irTOC1 Control 0 -33.1 14.5 112 -2.277 0.0247 *					
716 EV - irTOC1 Control 5 -29.8 14.5 112 -2.052 0.0425 *					
717 EV - irTOC1 W+OS 5 -5.6 14.5 112 -0.386 0.7002					
718 EV - irTOC1 Control 9 -39.0 14.5 112 -2.686 0.0083 **					
719 EV - irTOC1 W+OS 9 -16.6 14.5 112 -1.141 0.2565					
720 EV - irTOC1 Control 21 -60.2 14.5 112 -4.149 0.0001 ***					
721 EV - irTOC1 W+OS 21 -34.3 14.5 112 -2.361 0.0199 *					
722 EV - irTOC1 Control 33 -32.2 14.5 112 -2.216 0.0287 *					
723 EV - irTOC1 W+OS 33 -26.4 14.5 112 -1.820 0.0714 .					
724 EV - irTOC1 Control 45 -22.8 14.5 112 -1.571 0.1190					
725 EV - irTOC1 W+OS 45 -86.5 14.5 112 -5.961 <.0001 ***					
726 EV - irTOC1 Control 69 -37.2 14.5 112 -2.561 0.0118 *					
727 EV - irTOC1 W+OS 69 -113.8 14.5 112 -7.836 <.0001 ***					
728 EV - irTOC1 Control 93 -211.4 14.5 112 -14.556 <.0001 ***					
729	-----				
730 Caffeoylputrescine:					
731 ANOVA results for caffeoylputrescine concentration in ug/mL ("CP_ug_mL") as response variable:					
732 Response: CP_ug_mL					
733 Df Sum Sq Mean Sq F value Pr(>F)					

```

737 Genotype           1  348.5   348.5  415.1142 < 2.2e-16
738 ***
739 Treatment          1 3549.8  3549.8 4227.7746 < 2.2e-16
740 ***
741 Timepoint          7 2107.2   301.0  358.5205 < 2.2e-16
742 ***
743 Genotype:Treatment 1  114.2   114.2  136.0550 < 2.2e-16
744 ***
745 Genotype:Timepoint  7   50.9    7.3   8.6647  1.804e-08
746 ***
747 Treatment:Timepoint 5   664.2   132.8  158.2216 < 2.2e-16
748 ***
749 Genotype:Treatment:Timepoint 5   27.0    5.4   6.4304  2.735e-05
750 ***
751 Residuals          112  94.0    0.8
752 ---
753 Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
754 -----
755 EMMeans pairwise contrasts by treatment and time point in hours
756 ("Treatment", "Timepoint"):
757 contrast Treatment Timepoint estimate   SE  df t.ratio p.value
758 EV - irTOC1 Control    0            1.10 0.58 112  1.905 0.0593 .
759 EV - irTOC1 Control    5            1.15 0.58 112  1.981 0.0500 *
760 EV - irTOC1 W+OS       5            3.10 0.58 112  5.347 <.0001 ***
761 EV - irTOC1 Control    9            1.35 0.58 112  2.327 0.0217 *
762 EV - irTOC1 W+OS       9            3.75 0.58 112  6.479 <.0001 ***
763 EV - irTOC1 Control   21            1.04 0.58 112  1.795 0.0754 .
764 EV - irTOC1 W+OS      21            2.95 0.58 112  5.097 <.0001 ***
765 EV - irTOC1 Control   33            1.11 0.58 112  1.917 0.0578 .
766 EV - irTOC1 W+OS      33            3.46 0.58 112  5.965 <.0001 ***
767 EV - irTOC1 Control   45            0.62 0.58 112  1.070 0.2867
768 EV - irTOC1 W+OS      45            7.94 0.58 112 13.708 <.0001 ***
769 EV - irTOC1 Control   69            3.07 0.58 112  5.299 <.0001 ***
770 EV - irTOC1 W+OS      69            6.13 0.58 112 10.577 <.0001 ***
771 EV - irTOC1 W+OS      93            7.40 0.58 112 12.766 <.0001 ***
772
773 Dicaffeoylspermidine:
774 ANOVA results for dicaffeoylspermidine concentration in ug/mL
775 ("DCS_ug_mL") as response variable:
776 Response: DCS_ug_mL
777                               Df Sum Sq Mean Sq F value Pr(>F)
778 Genotype                   1 13602 13602.0 633.0032 < 2.2e-16 ***
779 Treatment                  1 20440 20440.3 951.2406 < 2.2e-16 ***
780 Timepoint                  7 42553 6079.0 282.9017 < 2.2e-16 ***
781 Genotype:Treatment         1   711   710.9  33.0854 7.773e-08 ***
782 Genotype:Timepoint         7   701   100.2   4.6616 0.0001298 ***
783 Treatment:Timepoint       5  9842 1968.4  91.6035 < 2.2e-16 ***
784 Genotype:Treatment:Timepoint 5 1092   218.4 10.1659 4.771e-08 ***

```

```

785 Residuals           112   2407    21.5
786 ---
787 Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
788 -----
789 EMMeans pairwise contrasts by treatment and time point in hours
790 ("Treatment","Timepoint"):
791 contrast Treatment Timepoint estimate   SE  df t.ratio p.value
792   EV - irTOC1 Control    0        11.8 2.93 112  4.025  0.0001 ***
793   EV - irTOC1 Control    5        11.5 2.93 112  3.916  0.0002 ***
794   EV - irTOC1 W+OS      5        26.4 2.93 112  8.996 <.0001 ***
795   EV - irTOC1 Control    9        27.4 2.93 112  9.343 <.0001 ***
796   EV - irTOC1 W+OS      9        15.3 2.93 112  5.206 <.0001 ***
797   EV - irTOC1 Control   21       15.6 2.93 112  5.330 <.0001 ***
798   EV - irTOC1 W+OS     21       27.1 2.93 112  9.252 <.0001 ***
799   EV - irTOC1 Control   33       13.6 2.93 112  4.636 <.0001 ***
800   EV - irTOC1 W+OS     33       14.1 2.93 112  4.821 <.0001 ***
801   EV - irTOC1 Control   45       10.3 2.93 112  3.518  0.0006 ***
802   EV - irTOC1 W+OS     45       29.4 2.93 112  10.029 <.0001 ***
803   EV - irTOC1 Control   69       16.2 2.93 112  5.541 <.0001 ***
804   EV - irTOC1 W+OS     69       39.9 2.93 112  13.614 <.0001 ***
805   EV - irTOC1 W+OS     93       17.3 2.93 112  5.913 <.0001 ***
806
807 Secondary metabolite 15N incorporation rates (Figure 3e,f,g):
808 Nicotine:
809 ANOVA results for nicotine 15N %At incorporation rate
810 ("Nic_15N_incorp") as response variable:
811 Response: Nic_15N_incorp
812                               Df Sum Sq Mean Sq F value    Pr(>F)
813 Genotype                  1  61.05  61.048 50.9327 1.044e-10 ***
814 Treatment                 1  23.06  23.064 19.2423 2.632e-05 ***
815 Timepoint                 7 366.31  52.330 43.6596 < 2.2e-16 ***
816 Genotype:Treatment        1   3.85   3.851  3.2128  0.075786 .
817 Genotype:Timepoint         7  24.73   3.533  2.9473  0.007188 **
818 Treatment:Timepoint       5 222.30  44.461 37.0939 < 2.2e-16 ***
819 Genotype:Treatment:Timepoint 5   5.99   1.198  0.9996  0.421510
820 Residuals                 111 133.04   1.199
821 ---
822 Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
823 -----
824 EMMeans pairwise contrasts by treatment and time point in hours
825 ("Treatment","Timepoint"):
826 contrast Treatment Timepoint estimate   SE  df t.ratio p.value
827   EV - irTOC1 Control    0        -1.48 0.692 111 -2.137  0.0348 *
828   EV - irTOC1 Control    5        -2.36 0.692 111 -3.408  0.0009 ***
829   EV - irTOC1 W+OS      5        -1.66 0.692 111 -2.397  0.0182 *
830   EV - irTOC1 Control    9        -2.54 0.692 111 -3.668  0.0004 ***
831   EV - irTOC1 W+OS      9        -2.92 0.692 111 -4.217  0.0001 ***

```

```

832   EV - irTOC1 Control    21      -0.84  0.734 111 -1.144  0.2552
833   EV - irTOC1 W+OS      21      -1.70  0.692 111 -2.455  0.0156 *
834   EV - irTOC1 Control    33      -0.88  0.692 111 -1.271  0.2064
835   EV - irTOC1 W+OS      33      -1.84  0.692 111 -2.657  0.0090 **
836   EV - irTOC1 Control    45       0.66  0.692 111  0.953  0.3426
837   EV - irTOC1 W+OS      45      -1.08  0.692 111 -1.560  0.1217
838   EV - irTOC1 Control    69       0.56  0.692 111  0.809  0.4204
839   EV - irTOC1 W+OS      69      -1.46  0.692 111 -2.109  0.0372 *
840   EV - irTOC1 W+OS      93      -0.88  0.692 111 -1.271  0.2064
841
842 Caffeoylputrescine:
843 ANOVA results for caffeoylputrescine 15N %At incorporation rate
844 ("CP_15N_incorp") as response variable:
845 Response: CP_15N_incorp
846
847 Genotype          Df  Sum Sq Mean Sq F value Pr(>F)
848 ***               1 1432.62 1432.62 931.3101 < 2.2e-16
849 Treatment         Df  Sum Sq Mean Sq F value Pr(>F)
850 ***               1 1330.78 1330.78 865.1058 < 2.2e-16
851 Timepoint         Df  Sum Sq Mean Sq F value Pr(>F)
852 ***               7 1954.63 279.23 181.5215 < 2.2e-16
853 Genotype:Treatment Df  Sum Sq Mean Sq F value Pr(>F)
854 ***               1    62.49   62.49  40.6255 4.334e-09
855 Genotype:Timepoint Df  Sum Sq Mean Sq F value Pr(>F)
856 ***               7   218.71   31.24  20.3107 < 2.2e-16
857 Treatment:Timepoint Df  Sum Sq Mean Sq F value Pr(>F)
858 ***               5    42.37    8.47   5.5092 0.0001437
859 Genotype:Treatment:Timepoint Df  Sum Sq Mean Sq F value Pr(>F)
860 ***               5   427.28   85.46  55.5526 < 2.2e-16
861 Residuals          111 170.75   1.54
862 ---
863 Signif. codes: 0 '****' 0.001 '***' 0.01 '**' 0.05 '*' 0.1 '.' 1
864 -----
865 EMMeans pairwise contrasts by treatment and time point in hours
866 ("Treatment", "Timepoint"):
867 contrast Treatment Timepoint estimate SE df t.ratio p.value
868   EV - irTOC1 Control 0 0.00 0.784 111 0.000 1.0000
869   EV - irTOC1 Control 5 0.00 0.784 111 0.000 1.0000
870   EV - irTOC1 W+OS 5 10.68 0.784 111 13.615 <.0001 ***
871   EV - irTOC1 Control 9 12.46 0.784 111 15.884 <.0001 ***
872   EV - irTOC1 W+OS 9 2.76 0.784 111 3.519 0.0006 ***
873   EV - irTOC1 Control 21 13.75 0.832 111 16.526 <.0001 ***
874   EV - irTOC1 W+OS 21 4.38 0.784 111 5.584 <.0001 ***
875   EV - irTOC1 Control 33 13.30 0.784 111 16.955 <.0001 ***
876   EV - irTOC1 W+OS 33 1.74 0.784 111 2.218 0.0286 *
877   EV - irTOC1 Control 45 8.80 0.784 111 11.218 <.0001 ***
878   EV - irTOC1 W+OS 45 4.14 0.784 111 5.278 <.0001 ***
879   EV - irTOC1 Control 69 6.92 0.784 111 8.822 <.0001 ***

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880   EV - irTOC1 W+OS      69          5.98 0.784 111  7.623 <.0001 ***
881   EV - irTOC1 W+OS      93          6.16 0.784 111  7.853 <.0001 ***
882
883 Dicaffeoylspermidine:
884 ANOVA results for dicaffeoylspermidine 15N %At incorporation rate
885 ("DCS_15N_incorp") as response variable:
886 Response: DCS_15N_incorp
887
888   Genotype             Df Sum Sq Mean Sq F value    Pr(>F)
889   Treatment            1  442.15 442.15 258.5097 < 2.2e-16 ***
890   Timepoint            7  65.83  65.83  38.4874 9.510e-09 ***
891   Genotype:Treatment   1  111.99 111.99 65.4780 < 2.2e-16 ***
892   Genotype:Timepoint   7  160.61 32.12  18.7808 1.607e-13 ***
893   Treatment:Timepoint 5   22.74  4.55   2.6595  0.02603 *
894   Genotype:Treatment:Timepoint 5   191.56 1.71
895   Residuals           112
896
897 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
898 -----
899 EMMeans pairwise contrasts by treatment and time point in hours
900 ("Treatment", "Timepoint"):
901 contrast Treatment Timepoint estimate     SE  df t.ratio p.value
902   EV - irTOC1 Control    0          1.74 0.827 112  2.104  0.0376 *
903   EV - irTOC1 Control    5          2.28 0.827 112  2.756  0.0068 **
904   EV - irTOC1 W+OS      5          3.32 0.827 112  4.014  0.0001 ***
905   EV - irTOC1 Control    9          4.94 0.827 112  5.972 <.0001 ***
906   EV - irTOC1 W+OS      9          2.26 0.827 112  2.732  0.0073 **
907   EV - irTOC1 Control   21         5.38 0.827 112  6.504 <.0001 ***
908   EV - irTOC1 W+OS     21         5.46 0.827 112  6.601 <.0001 ***
909   EV - irTOC1 Control   33         3.70 0.827 112  4.473 <.0001 ***
910   EV - irTOC1 W+OS     33         3.02 0.827 112  3.651  0.0004 ***
911   EV - irTOC1 Control   45         3.40 0.827 112  4.111  0.0001 ***
912   EV - irTOC1 W+OS     45         6.08 0.827 112  7.351 <.0001 ***
913   EV - irTOC1 Control   69         3.74 0.827 112  4.522 <.0001 ***
914   EV - irTOC1 W+OS     69         2.16 0.827 112  2.611  0.0103 *
915   EV - irTOC1 W+OS     93         2.28 0.827 112  2.756  0.0068 **
916
917
918 15N-labeled secondary metabolite concentrations (Figure 3h,j,k):
919 Nicotine:
920 ANOVA results for labeled nicotine concentration in ug/mL ("Nic_15N")
921 as response variable:
922 Response: Nic_15N
923
924   Genotype             Df Sum Sq Mean Sq F value    Pr(>F)
925   Treatment            1  4053.3 4053.3 193.2173 < 2.2e-16 ***
926   Timepoint            7  7179.7 7179.7 342.2481 < 2.2e-16 ***

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927 Genotype:Treatment           1  523.6   523.6  24.9606 2.196e-06 ***
928 Genotype:Timepoint            7  722.7   103.2   4.9214 7.184e-05 ***
929 Treatment:Timepoint          5 2266.9   453.4   21.6125 4.706e-15 ***
930 Genotype:Treatment:Timepoint 5  372.5    74.5    3.5517  0.005122 **
931 Residuals                   111 2328.6   21.0
932 ---
933 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
934 -----
935 EMMeans pairwise contrasts by treatment and time point in hours
936 ("Treatment", "Timepoint"):
937 contrast Treatment Timepoint estimate SE df t.ratio p.value
938 EV - irTOC1 Control 0      -5.73 2.90 111 -1.978 0.0504 .
939 EV - irTOC1 Control 5      -9.65 2.90 111 -3.331 0.0012 **
940 EV - irTOC1 W+OS 5      -6.29 2.90 111 -2.173 0.0319 *
941 EV - irTOC1 Control 9      -12.46 2.90 111 -4.300 <.0001 ***
942 EV - irTOC1 W+OS 9      -11.62 2.90 111 -4.012 0.0001 ***
943 EV - irTOC1 Control 21     -9.15 3.07 111 -2.980 0.0035 **
944 EV - irTOC1 W+OS 21     -9.75 2.90 111 -3.366 0.0010 ***
945 EV - irTOC1 Control 33     -5.74 2.90 111 -1.981 0.0501 .
946 EV - irTOC1 W+OS 33     -10.67 2.90 111 -3.684 0.0004 ***
947 EV - irTOC1 Control 45     -1.17 2.90 111 -0.404 0.6869
948 EV - irTOC1 W+OS 45     -15.51 2.90 111 -5.353 <.0001 ***
949 EV - irTOC1 Control 69     -4.01 2.90 111 -1.386 0.1687
950 EV - irTOC1 W+OS 69     -18.44 2.90 111 -6.365 <.0001 ***
951 EV - irTOC1 W+OS 93     -29.40 2.90 111 -10.148 <.0001 ***
952
953 Caffeoylputrescine:
954 ANOVA results for labeled caffeoylputrescine concentration in ug/mL
955 ("CP_15N") as response variable:
956 Response: CP_15N
957
958 Genotype Df Sum Sq Mean Sq F value Pr(>F)
959 Treatment 1 19.874 19.874 900.120 < 2.2e-16 ***
960 Timepoint 7 63.113 9.016 408.349 < 2.2e-16 ***
961 Genotype:Treatment 1 9.144 9.144 414.156 < 2.2e-16 ***
962 Genotype:Timepoint 7 7.645 1.092 49.465 < 2.2e-16 ***
963 Treatment:Timepoint 5 18.637 3.727 168.818 < 2.2e-16 ***
964 Genotype:Treatment:Timepoint 5 1.952 0.390 17.680 7.458e-13 ***
965 Residuals 111 2.451 0.022
966 ---
967 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
968 -----
969 EMMeans pairwise contrasts by treatment and time point in hours
970 ("Treatment", "Timepoint"):
971 contrast Treatment Timepoint estimate SE df t.ratio p.value
972 EV - irTOC1 Control 0      0.000 0.0940 111 0.000 1.0000
973 EV - irTOC1 Control 5      0.000 0.0940 111 0.000 1.0000

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```

974   EV - irTOC1 W+OS      5          0.664 0.0940 111  7.068 <.0001
975   ***
976   EV - irTOC1 Control    9          0.271 0.0940 111  2.887 0.0047 **
977   EV - irTOC1 W+OS      9          0.561 0.0940 111  5.968 <.0001
978   ***
979   EV - irTOC1 Control    21         0.353 0.0997 111  3.537 0.0006
980   ***
981   EV - irTOC1 W+OS      21         0.931 0.0940 111  9.912 <.0001
982   ***
983   EV - irTOC1 Control    33         0.286 0.0940 111  3.044 0.0029 **
984   EV - irTOC1 W+OS      33         0.565 0.0940 111  6.012 <.0001
985   ***
986   EV - irTOC1 Control    45         0.235 0.0940 111  2.498 0.0139 *
987   EV - irTOC1 W+OS      45         1.773 0.0940 111  18.865 <.0001
988   ***
989   EV - irTOC1 Control    69         0.627 0.0940 111  6.673 <.0001
990   ***
991   EV - irTOC1 W+OS      69         2.075 0.0940 111  22.083 <.0001
992   ***
993   EV - irTOC1 W+OS      93         2.355 0.0940 111  25.057 <.0001
994   ***
995
996 Dicaffeoylspermidine:
997 ANOVA results for labeled dicaffeoylspermidine concentration in ug/mL
998 ("DCS_15N") as response variable:
999 Response: DCS_15N
1000
1001 Genotype             Df Sum Sq Mean Sq F value    Pr(>F)
1002 Treatment            1  261.29 261.293 373.061 < 2.2e-16 ***
1003 Timepoint            1  275.94 275.945 393.979 < 2.2e-16 ***
1004 Timepoint             7  777.78 111.111 158.639 < 2.2e-16 ***
1005 Genotype:Treatment   1   25.27  25.273  36.084 2.397e-08 ***
1006 Genotype:Timepoint   7   49.26   7.037  10.047 1.063e-09 ***
1007 Treatment:Timepoint  5  185.61  37.121  53.000 < 2.2e-16 ***
1008 Treatment:Treatment:Timepoint  5   39.19   7.839  11.191 9.220e-09 ***
1009 Residuals           112  78.45   0.700
1010 ---
1011 Signif. codes:  0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1012 -----
1013 EMMeans pairwise contrasts by treatment and time point in hours
1014 ("Treatment", "Timepoint"):
1015 contrast Treatment Timepoint estimate     SE   df t.ratio p.value
1016   EV - irTOC1 Control 0          0.560 0.529 112  1.058 0.2923
1017   EV - irTOC1 Control 5          0.832 0.529 112  1.573 0.1186
1018   EV - irTOC1 W+OS   5          2.265 0.529 112  4.279 <.0001 ***
1019   EV - irTOC1 Control 9          2.805 0.529 112  5.299 <.0001 ***
1020   EV - irTOC1 W+OS   9          1.200 0.529 112  2.267 0.0253 *
1021   EV - irTOC1 Control 21         2.512 0.529 112  4.746 <.0001 ***
1022   EV - irTOC1 W+OS   21         3.625 0.529 112  6.848 <.0001 ***

```

1022 EV - irTOC1 Control 33 1.881 0.529 112 3.553 0.0006 ***

 1023 EV - irTOC1 W+OS 33 1.191 0.529 112 2.251 0.0263 *

 1024 EV - irTOC1 Control 45 1.784 0.529 112 3.370 0.0010 ***

 1025 EV - irTOC1 W+OS 45 7.131 0.529 112 13.472 <.0001 ***

 1026 EV - irTOC1 Control 69 2.804 0.529 112 5.298 <.0001 ***

 1027 EV - irTOC1 W+OS 69 5.721 0.529 112 10.809 <.0001 ***

 1028 EV - irTOC1 W+OS 93 3.941 0.529 112 7.446 <.0001 ***

 1029

1030 Secondary metabolite total pools (Figure S1b,c,d):
Nicotine:
 1032 ANOVA results for whole-leaf nicotine pools ("Nic_total_pools") as
 1033 response variable:
 1034 Response: Nic_total_pools
 1035 Df Sum Sq Mean Sq F value Pr(>F)
 1036 Genotype 1 546217 546217 69.2627 2.346e-13
 1037 ***
 1038 Treatment 1 1479607 1479607 187.6207 < 2.2e-16
 1039 ***
 1040 Timepoint 7 2863564 409081 51.8732 < 2.2e-16
 1041 ***
 1042 Genotype:Treatment 1 37748 37748 4.7867 0.03076 *
 1043 Genotype:Timepoint 7 267491 38213 4.8456 8.463e-05
 1044 ***
 1045 Treatment:Timepoint 5 310473 62095 7.8739 2.197e-06
 1046 ***
 1047 Genotype:Treatment:Timepoint 5 150131 30026 3.8075 0.00319 **
 1048 Residuals 112 883250 7886
 1049 ---
 1050 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
 1051 -----
 1052 EMMeans pairwise contrasts by treatment and time point in hours
 1053 ("Treatment", "Timepoint"):
 1054 contrast Treatment Timepoint estimate SE df t.ratio p.value
 1055 EV - irTOC1 Control 0 -24.4 56.2 112 -0.435 0.6645
 1056 EV - irTOC1 Control 5 -42.1 56.2 112 -0.750 0.4550
 1057 EV - irTOC1 W+OS 5 -29.4 56.2 112 -0.524 0.6016
 1058 EV - irTOC1 Control 9 -45.5 56.2 112 -0.810 0.4194
 1059 EV - irTOC1 W+OS 9 -64.2 56.2 112 -1.143 0.2555
 1060 EV - irTOC1 Control 21 -258.7 56.2 112 -4.606 <.0001 ***
 1061 EV - irTOC1 W+OS 21 -20.2 56.2 112 -0.359 0.7202
 1062 EV - irTOC1 Control 33 -62.5 56.2 112 -1.113 0.2679
 1063 EV - irTOC1 W+OS 33 -85.0 56.2 112 -1.513 0.1330
 1064 EV - irTOC1 Control 45 -121.0 56.2 112 -2.155 0.0333 *
 1065 EV - irTOC1 W+OS 45 -199.8 56.2 112 -3.557 0.0006 ***
 1066 EV - irTOC1 Control 69 -90.3 56.2 112 -1.607 0.1108
 1067 EV - irTOC1 W+OS 69 -331.1 56.2 112 -5.896 <.0001 ***
 1068 EV - irTOC1 W+OS 93 -374.7 56.2 112 -6.671 <.0001 ***

```

1069
1070 Caffeoylputrescine:
1071 ANOVA results for whole-leaf caffeoylputrescine pools
1072 ("CP_total_pools") as response variable:
1073 Response: CP_total_pools
1074
1075 Genotype Df Sum Sq Mean Sq F value Pr(>F)
1076 *** 1 1076.1 1076.1 150.2229 < 2.2e-16
1077 Treatment 1 12834.3 12834.3 1791.6866 < 2.2e-16
1078 ***
1079 Timepoint 7 12202.0 1743.1 243.3444 < 2.2e-16
1080 ***
1081 Genotype:Treatment 1 393.1 393.1 54.8782 2.584e-11
1082 ***
1083 Genotype:Timepoint 7 422.9 60.4 8.4347 2.925e-08
1084 ***
1085 Treatment:Timepoint 5 3564.2 712.8 99.5131 < 2.2e-16
1086 ***
1087 Genotype:Treatment:Timepoint 5 141.4 28.3 3.9470 0.002468
1088 **
1089 Residuals 112 802.3 7.2
1090 ---
1091 Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1092 -----
1093 EMMeans pairwise contrasts by treatment and time point in hours
1094 ("Treatment", "Timepoint"):
1095 contrast Treatment Timepoint estimate SE df t.ratio p.value
1096 EV - irTOC1 Control 0 1.281 1.69 112 0.757 0.4509
1097 EV - irTOC1 Control 5 1.822 1.69 112 1.076 0.2841
1098 EV - irTOC1 W+OS 5 3.921 1.69 112 2.316 0.0224 *
1099 EV - irTOC1 Control 9 2.201 1.69 112 1.300 0.1962
1100 EV - irTOC1 W+OS 9 4.215 1.69 112 2.490 0.0142 *
1101 EV - irTOC1 Control 21 0.182 1.69 112 0.107 0.9146
1102 EV - irTOC1 W+OS 21 5.797 1.69 112 3.425 0.0009 ***
1103 EV - irTOC1 Control 33 1.898 1.69 112 1.121 0.2645
1104 EV - irTOC1 W+OS 33 5.467 1.69 112 3.230 0.0016 **
1105 EV - irTOC1 Control 45 0.462 1.69 112 0.273 0.7853
1106 EV - irTOC1 W+OS 45 14.777 1.69 112 8.730 <.0001 ***
1107 EV - irTOC1 Control 69 7.508 1.69 112 4.436 <.0001 ***
1108 EV - irTOC1 W+OS 69 9.944 1.69 112 5.875 <.0001 ***
1109 EV - irTOC1 W+OS 93 18.152 1.69 112 10.723 <.0001 ***
1110
1111 Dicaffeoylspermidine:
1112 ANOVA results for whole-leaf dicaffeoylspermidine pools
1113 ("DCS_total_pools") as response variable:
1114 Response: DCS_total_pools
1115
1116 Genotype Df Sum Sq Mean Sq F value Pr(>F)

```

```

1117 Treatment 1 87931 87931 426.8139 < 2.2e-16 ***
1118 Timepoint 7 285038 40720 197.6511 < 2.2e-16 ***
1119 Genotype:Treatment 1 3377 3377 16.3935 9.508e-05 ***
1120 Genotype:Timepoint 7 4497 642 3.1181 0.0048106 **
1121 Treatment:Timepoint 5 48778 9756 47.3528 < 2.2e-16 ***
1122 Genotype:Treatment:Timepoint 5 4973 995 4.8277 0.0004912 ***
1123 Residuals 112 23074 206
1124 ---
1125 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1126 -----
1127 EMMeans pairwise contrasts by treatment and time point in hours
("Treatment", "Timepoint"):
1128 contrast Treatment Timepoint estimate SE df t.ratio p.value
1129 EV - irTOC1 Control 0 14.28 9.08 112 1.573 0.1186
1130 EV - irTOC1 Control 5 18.55 9.08 112 2.043 0.0434 *
1131 EV - irTOC1 W+OS 5 33.57 9.08 112 3.698 0.0003 ***
1132 EV - irTOC1 Control 9 44.66 9.08 112 4.919 <.0001 ***
1133 EV - irTOC1 W+OS 9 16.66 9.08 112 1.836 0.0691 .
1134 EV - irTOC1 Control 21 6.50 9.08 112 0.717 0.4752
1135 EV - irTOC1 W+OS 21 43.41 9.08 112 4.782 <.0001 ***
1136 EV - irTOC1 Control 33 23.78 9.08 112 2.620 0.0100 **
1137 EV - irTOC1 W+OS 33 21.99 9.08 112 2.423 0.0170 *
1138 EV - irTOC1 Control 45 8.39 9.08 112 0.925 0.3571
1139 EV - irTOC1 W+OS 45 56.06 9.08 112 6.175 <.0001 ***
1140 EV - irTOC1 Control 69 41.22 9.08 112 4.540 <.0001 ***
1141 EV - irTOC1 W+OS 69 74.36 9.08 112 8.192 <.0001 ***
1142 EV - irTOC1 Control 93 48.84 9.08 112 5.381 <.0001 ***
1143 EV - irTOC1 W+OS
1144
1145 Secondary metabolite total 15N-labeled pools (Figure S1e,f,g):
1146 Nicotine:
1147 ANOVA results for whole-leaf 15N-labeled nicotine pools
1148 ("Nic_15N_pools") as response variable:
1149 Response: Nic_15N_pools
1150 Df Sum Sq Mean Sq F value Pr(>F)
1151 Genotype 1 17394 17394.2 125.1303 < 2.2e-16 ***
1152 Treatment 1 25104 25103.9 180.5923 < 2.2e-16 ***
1153 Timepoint 7 61616 8802.3 63.3219 < 2.2e-16 ***
1154 Genotype:Treatment 1 1870 1869.9 13.4516 0.0003775 ***
1155 Genotype:Timepoint 7 2721 388.8 2.7966 0.0101831 *
1156 Treatment:Timepoint 5 12837 2567.3 18.4687 2.603e-13 ***
1157 Genotype:Treatment:Timepoint 5 3043 608.6 4.3784 0.0011239 **
1158 Residuals 111 15430 139.0
1159 ---
1160 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1161 -----
1162 EMMeans pairwise contrasts by treatment and time point in hours
("Treatment", "Timepoint"):

```

```

1164 contrast Treatment Timepoint estimate SE df t.ratio p.value
1165 EV - irTOC1 Control 0 -5.64 7.46 111 -0.756 0.4511
1166 EV - irTOC1 Control 5 -14.62 7.46 111 -1.960 0.0525 .
1167 EV - irTOC1 W+OS 5 -10.83 7.46 111 -1.453 0.1490
1168 EV - irTOC1 Control 9 -17.40 7.46 111 -2.334 0.0214 *
1169 EV - irTOC1 W+OS 9 -21.61 7.46 111 -2.898 0.0045 **
1170 EV - irTOC1 Control 21 -35.44 7.91 111 -4.481 <.0001 ***
1171 EV - irTOC1 W+OS 21 -10.66 7.46 111 -1.430 0.1555
1172 EV - irTOC1 Control 33 -10.28 7.46 111 -1.378 0.1709
1173 EV - irTOC1 W+OS 33 -24.50 7.46 111 -3.286 0.0014 **
1174 EV - irTOC1 Control 45 -12.58 7.46 111 -1.687 0.0945 .
1175 EV - irTOC1 W+OS 45 -34.53 7.46 111 -4.631 <.0001 ***
1176 EV - irTOC1 Control 69 -9.63 7.46 111 -1.291 0.1993
1177 EV - irTOC1 W+OS 69 -49.93 7.46 111 -6.696 <.0001 ***
1178 EV - irTOC1 W+OS 93 -53.08 7.46 111 -7.118 <.0001 ***
1179
1180 Caffeoylputrescine:
1181 ANOVA results for whole-leaf 15N-labeled caffeoylputrescine pools
1182 ("CP_15N_pools") as response variable:
1183 Response: CP_15N_pools
1184
1185 Genotype Df Sum Sq Mean Sq F value Pr(>F)
1186 Treatment 1 69.32 69.315 534.034 < 2.2e-16 ***
1187 Timepoint 7 333.29 47.614 366.833 < 2.2e-16 ***
1188 Genotype:Treatment 1 32.52 32.516 250.517 < 2.2e-16 ***
1189 Genotype:Timepoint 7 46.58 6.655 51.270 < 2.2e-16 ***
1190 Treatment:Timepoint 5 96.80 19.359 149.153 < 2.2e-16 ***
1191 Genotype:Treatment:Timepoint 5 8.46 1.692 13.037 5.535e-10 ***
1192 Residuals 111 14.41 0.130
1193 ---
1194 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1195 -----
1196 EMMeans pairwise contrasts by treatment and time point in hours
1197 ("Treatment", "Timepoint"):
1198 contrast Treatment Timepoint estimate SE df t.ratio p.value
1199 EV - irTOC1 Control 0 0.000 0.228 111 0.000 1.0000
1200 EV - irTOC1 Control 5 0.000 0.228 111 0.000 1.0000
1201 EV - irTOC1 W+OS 5 0.886 0.228 111 3.889 0.0002 ***
1202 EV - irTOC1 Control 9 0.434 0.228 111 1.906 0.0592 .
1203 EV - irTOC1 W+OS 9 0.680 0.228 111 2.982 0.0035 **
1204 EV - irTOC1 Control 21 0.403 0.242 111 1.667 0.0982 .
1205 EV - irTOC1 W+OS 21 1.520 0.228 111 6.670 <.0001 ***
1206 EV - irTOC1 Control 33 0.485 0.228 111 2.130 0.0354 *
1207 EV - irTOC1 W+OS 33 0.910 0.228 111 3.994 0.0001 ***
1208 EV - irTOC1 Control 45 0.332 0.228 111 1.456 0.1483
1209 EV - irTOC1 W+OS 45 3.415 0.228 111 14.988 <.0001 ***
1210 EV - irTOC1 Control 69 1.557 0.228 111 6.834 <.0001 ***
1211 EV - irTOC1 W+OS 69 4.103 0.228 111 18.005 <.0001 ***

```

```

1212 EV - irTOC1 W+OS      93          5.263 0.228 111 23.100 <.0001 ***
1213
1214 Dicaffeoylspermidine:
1215 ANOVA results for whole-leaf 15N-labeled dicaffeoylspermidine pools
1216 ("DCS_15N_pools") as response variable:
1217 Response: DCS_15N_pools
1218                               Df Sum Sq Mean Sq F value    Pr(>F)
1219 Genotype                  1  838.3  838.25 197.4213 < 2.2e-16 ***
1220 Treatment                 1 1208.9 1208.87 284.7057 < 2.2e-16 ***
1221 Timepoint                 7 4079.9  582.84 137.2683 < 2.2e-16 ***
1222 Genotype:Treatment        1   111.8   111.82  26.3349 1.218e-06 ***
1223 Genotype:Timepoint         7   276.2    39.46   9.2924 4.913e-09 ***
1224 Treatment:Timepoint       5   830.5   166.09  39.1174 < 2.2e-16 ***
1225 Genotype:Treatment:Timepoint 5   163.7    32.74   7.7099 2.914e-06 ***
1226 Residuals                112  475.6     4.25
1227 ---
1228 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1229 -----
1230 EMMeans pairwise contrasts by treatment and time point in hours
1231 ("Treatment", "Timepoint"):
1232 contrast Treatment Timepoint estimate SE df t.ratio p.value
1233 EV - irTOC1 Control    0        0.669 1.3 112  0.513  0.6087
1234 EV - irTOC1 Control    5        1.294 1.3 112  0.993  0.3229
1235 EV - irTOC1 W+OS      5        2.874 1.3 112  2.206  0.0295 *
1236 EV - irTOC1 Control    9        4.439 1.3 112  3.406  0.0009 ***
1237 EV - irTOC1 W+OS      9        1.527 1.3 112  1.172  0.2439
1238 EV - irTOC1 Control   21       2.462 1.3 112  1.889  0.0615 .
1239 EV - irTOC1 W+OS      21       5.497 1.3 112  4.218  0.0001 ***
1240 EV - irTOC1 Control   33       3.332 1.3 112  2.557  0.0119 *
1241 EV - irTOC1 W+OS      33       2.014 1.3 112  1.545  0.1251
1242 EV - irTOC1 Control   45       2.494 1.3 112  1.914  0.0582 .
1243 EV - irTOC1 W+OS      45      14.108 1.3 112 10.826 <.0001 ***
1244 EV - irTOC1 Control   69       7.056 1.3 112  5.414  <.0001 ***
1245 EV - irTOC1 W+OS      69      11.331 1.3 112  8.694  <.0001 ***
1246 EV - irTOC1 W+OS      93      9.418 1.3 112  7.226  <.0001 ***
1247
1248
1249 Normalized differences in concentration vs. Nicotine (Figure 4c,d):
1250 Nicotine vs. caffeoylputrescine:
1251 ANOVA results for normalized differences in concentration between
1252 nicotine and caffeoylputrescine ("Norm_Nic_CP_conc") as response
1253 variable:
1254 Response: Norm_Nic_CP_conc
1255                               Df Sum Sq Mean Sq F value    Pr(>F)
1256 Genotype                  1  0.023170 0.023170 494.4796 < 2.2e-16
1257 ***

```

```

1258 Treatment 1 0.085159 0.085159 1817.4079 < 2.2e-16
1259 ***
1260 Timepoint 7 0.050061 0.007152 152.6242 < 2.2e-16
1261 ***
1262 Genotype:Treatment 1 0.006783 0.006783 144.7648 < 2.2e-16
1263 ***
1264 Genotype:Timepoint 7 0.007433 0.001062 22.6622 < 2.2e-16
1265 ***
1266 Treatment:Timepoint 5 0.013925 0.002785 59.4348 < 2.2e-16
1267 ***
1268 Genotype:Treatment:Timepoint 5 0.001593 0.000319 6.7999 1.423e-05
1269 ***
1270 Residuals 112 0.005248 0.000047
1271 ---
1272 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1273 -----
1274 EMeans pairwise contrasts by treatment and time point in hours
1275 ("Treatment", "Timepoint"):
1276 contrast Treatment Timepoint estimate SE df t.ratio p.value
1277 EV - irTOC1 Control 0 -0.00993 0.00433 112 -2.294 0.0237 *
1278 EV - irTOC1 Control 5 -0.00951 0.00433 112 -2.196 0.0301 *
1279 EV - irTOC1 W+OS 5 -0.01826 0.00433 112 -4.219 0.0001
1280 ***
1281 EV - irTOC1 Control 9 -0.01122 0.00433 112 -2.592 0.0108 *
1282 EV - irTOC1 W+OS 9 -0.02401 0.00433 112 -5.546 <.0001
1283 ***
1284 EV - irTOC1 Control 21 -0.01100 0.00433 112 -2.541 0.0124 *
1285 EV - irTOC1 W+OS 21 -0.02364 0.00433 112 -5.461 <.0001
1286 ***
1287 EV - irTOC1 Control 33 -0.01014 0.00433 112 -2.342 0.0209 *
1288 EV - irTOC1 W+OS 33 -0.02131 0.00433 112 -4.923 <.0001
1289 ***
1290 EV - irTOC1 Control 45 -0.00562 0.00433 112 -1.297 0.1973
1291 EV - irTOC1 W+OS 45 -0.05480 0.00433 112 -12.659 <.0001
1292 ***
1293 EV - irTOC1 Control 69 -0.02524 0.00433 112 -5.830 <.0001
1294 ***
1295 EV - irTOC1 W+OS 69 -0.05605 0.00433 112 -12.946 <.0001
1296 ***
1297 EV - irTOC1 W+OS 93 -0.07948 0.00433 112 -18.358 <.0001
1298 ***
1299
1300 Nicotine vs. dicaffeoylspermidine:
1301 ANOVA results for normalized differences in concentration between
1302 nicotine and dicaffeoylspermidine ("Norm_Nic_DCS_conc") as response
1303 variable:
1304 Response: Norm_Nic_DCS_conc
1305 Df Sum Sq Mean Sq F value Pr(>F)

```

```

1306 Genotype           1 0.61560 0.61560 767.6298 < 2.2e-16
1307 ***
1308 Treatment          1 0.09933 0.09933 123.8544 < 2.2e-16
1309 ***
1310 Timepoint          7 0.61212 0.08745 109.0417 < 2.2e-16
1311 ***
1312 Genotype:Treatment 1 0.01056 0.01056 13.1729 0.0004295
1313 ***
1314 Genotype:Timepoint 7 0.02848 0.00407 5.0739 4.988e-05
1315 ***
1316 Treatment:Timepoint 5 0.12672 0.02534 31.6035 < 2.2e-16
1317 ***
1318 Genotype:Treatment:Timepoint 5 0.03691 0.00738 9.2041 2.318e-07
1319 ***
1320 Residuals          112 0.08982 0.00080
1321 ---
1322 Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1323 -----
1324 EMMeans pairwise contrasts by treatment and time point in hours
1325 ("Treatment", "Timepoint") :
1326 contrast Treatment Timepoint estimate      SE   df t.ratio p.value
1327 EV - irTOC1 Control    0        -0.0997 0.0179 112  -5.565 <.0001
1328 ***
1329 EV - irTOC1 Control    5        -0.0905 0.0179 112  -5.055 <.0001
1330 ***
1331 EV - irTOC1 W+OS       5        -0.1316 0.0179 112  -7.348 <.0001
1332 ***
1333 EV - irTOC1 Control    9        -0.1834 0.0179 112 -10.238 <.0001
1334 ***
1335 EV - irTOC1 W+OS       9        -0.0867 0.0179 112  -4.841 <.0001
1336 ***
1337 EV - irTOC1 Control   21        -0.1313 0.0179 112  -7.332 <.0001
1338 ***
1339 EV - irTOC1 W+OS       21        -0.1480 0.0179 112  -8.265 <.0001
1340 ***
1341 EV - irTOC1 Control   33        -0.1082 0.0179 112  -6.041 <.0001
1342 ***
1343 EV - irTOC1 W+OS       33        -0.0797 0.0179 112  -4.448 <.0001
1344 ***
1345 EV - irTOC1 Control   45        -0.0757 0.0179 112  -4.227 <.0001
1346 ***
1347 EV - irTOC1 W+OS       45        -0.1702 0.0179 112  -9.503 <.0001
1348 ***
1349 EV - irTOC1 Control   69        -0.1180 0.0179 112  -6.587 <.0001
1350 ***
1351 EV - irTOC1 W+OS       69        -0.2251 0.0179 112 -12.567 <.0001
1352 ***

```

```

1353 EV - irTOC1 W+OS      93      -0.2087 0.0179 112 -11.651 <.0001
1354 ***
1355
1356 Normalized differences in 15N incorporation vs. nicotine (Figure 4e,f):
1357 Nicotine vs. caffeoylputrescine:
1358 ANOVA results for normalized differences in incorporation rates
1359 between nicotine and caffeoylputrescine ("Norm_Nic_CP_15N_incorp") as
1360 response variable:
1361 Response: Norm_Nic_CP_15N_incorp
1362
1363   Df Sum Sq Mean Sq F value Pr(>F)
1364 Genotype          1 6.4892 6.4892 1683.518 < 2.2e-16
1365 ***
1366 Treatment         1 7.5818 7.5818 1966.984 < 2.2e-16
1367 ***
1368 Timepoint         7 10.1727 1.4532 377.023 < 2.2e-16
1369 ***
1370 Genotype:Treatment 1 0.4213 0.4213 109.310 < 2.2e-16
1371 ***
1372 Genotype:Timepoint 7 1.6013 0.2288 59.349 < 2.2e-16
1373 ***
1374 Treatment:Timepoint 5 0.2299 0.0460 11.930 3.001e-09
1375 ***
1376 Genotype:Treatment:Timepoint 5 3.1248 0.6250 162.134 < 2.2e-16
1377 Residuals          111 0.4279 0.0039
1378 ---
1379 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1380 -----
1381 EMMeans pairwise contrasts by treatment and time point in hours
1382 ("Treatment", "Timepoint"):
1383 contrast Treatment Timepoint estimate     SE df t.ratio p.value
1384 EV - irTOC1 Control    0      0.000 0.0393 111  0.000 1.0000
1385 EV - irTOC1 Control    5      0.000 0.0393 111  0.000 1.0000
1386 EV - irTOC1 W+OS      5     -0.931 0.0393 111 -23.713 <.0001
1387 ***
1388 EV - irTOC1 Control    9     -0.999 0.0393 111 -25.446 <.0001
1389 ***
1390 EV - irTOC1 W+OS      9     -0.260 0.0393 111 -6.627 <.0001
1391 ***
1392 EV - irTOC1 Control   21     -1.088 0.0416 111 -26.123 <.0001
1393 ***
1394 EV - irTOC1 W+OS      21     -0.266 0.0393 111 -6.774 <.0001
1395 ***
1396 EV - irTOC1 Control   33     -1.092 0.0393 111 -27.798 <.0001
1397 ***
1398 EV - irTOC1 W+OS      33     -0.121 0.0393 111 -3.075 0.0027 **

```

```

1399   EV - irTOC1 Control    45      -0.412 0.0393 111 -10.489 <.0001
1400 ***
1401   EV - irTOC1 W+OS      45      -0.183 0.0393 111 -4.656 <.0001
1402 ***
1403   EV - irTOC1 Control    69      -0.286 0.0393 111 -7.293 <.0001
1404 ***
1405   EV - irTOC1 W+OS      69      -0.269 0.0393 111 -6.860 <.0001
1406 ***
1407   EV - irTOC1 W+OS      93      -0.240 0.0393 111 -6.118 <.0001
1408 ***
1409
1410 Nicotine vs. dicaffeoylspermidine:
1411 ANOVA results for normalized differences in incorporation rates
1412 between nicotine and dicaffeoylspermidine ("Norm_Nic_DCS_15N_incorp")
1413 as response variable:
1414 Response: Norm_Nic_DCS_15N_incorp
1415
1416   Df Sum Sq Mean Sq F value Pr(>F)
1417 Genotype          1 3.4671 3.4671 245.6765 < 2.2e-16 ***
1418 Treatment         1 0.1681 0.1681 11.9133 0.0007894 ***
1419 Timepoint         7 3.2123 0.4589 32.5172 < 2.2e-16 ***
1420 Genotype:Treatment 1 0.0088 0.0088 0.6252 0.4308100
1421 Genotype:Timepoint 7 0.3125 0.0446 3.1638 0.0043447 **
1422 Treatment:Timepoint 5 1.5519 0.3104 21.9938 2.947e-15 ***
1423 Genotype:Treatment:Timepoint 5 0.0822 0.0164 1.1654 0.3307764
1424 Residuals          111 1.5665 0.0141
1425 ---
1426 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1427 -----
1428 EMMeans pairwise contrasts by treatment and time point in hours
1429 ("Treatment","Timepoint"):
1430 contrast Treatment Timepoint estimate     SE df t.ratio p.value
1431   EV - irTOC1 Control    0      -0.376 0.0751 111 -5.006 <.0001
1432 ***
1433   EV - irTOC1 Control    5      -0.343 0.0751 111 -4.568 <.0001
1434 ***
1435   EV - irTOC1 W+OS      5      -0.333 0.0751 111 -4.428 <.0001
1436 ***
1437   EV - irTOC1 Control    9      -0.496 0.0751 111 -6.596 <.0001
1438 ***
1439   EV - irTOC1 W+OS      9      -0.309 0.0751 111 -4.107 0.0001
1440 ***
1441   EV - irTOC1 Control   21      -0.409 0.0797 111 -5.128 <.0001
1442 ***
1443   EV - irTOC1 W+OS      21      -0.503 0.0751 111 -6.700 <.0001
1444 ***
1445   EV - irTOC1 Control   33      -0.288 0.0751 111 -3.837 0.0002
1446 ***

```

```

1446 EV - irTOC1 W+OS      33      -0.291 0.0751 111 -3.869 0.0002
1447 ***
1448 EV - irTOC1 Control    45      -0.200 0.0751 111 -2.665 0.0089 **
1449 EV - irTOC1 W+OS      45      -0.347 0.0751 111 -4.614 <.0001
1450 ***
1451 EV - irTOC1 Control    69      -0.206 0.0751 111 -2.742 0.0071 **
1452 EV - irTOC1 W+OS      69      -0.177 0.0751 111 -2.353 0.0204 *
1453 EV - irTOC1 W+OS      93      -0.139 0.0751 111 -1.856 0.0661 .
1454

```

1455 **Figure 5:**

1456 Outlier removal:

1457 Data from only one DCS data point (root W+OS, irTOC1 replicate 5) was
1458 removed after being identified using our outlier procedure; this peak
1459 was ~15x the magnitude of other peaks in its cohort (genotype and
1460 treatment), thus removed from the analysis. Re-analysis of the data
1461 results with the data point did not change the statistical results,
1462 which were insignificant for genotype or treatment for DCS levels in
1463 the roots.

1464 Secondary metabolites (leaves):

1465 **Nicotine:**

1466 ANOVA results for nicotine concentration ("value") as response
1467 variable:

1468 Response: value

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	1	2.1170e+13	2.1170e+13	6.2691	0.02349 *
Treatment	1	6.0867e+12	6.0867e+12	1.8025	0.19815
Genotype:Treatment	1	2.8104e+12	2.8104e+12	0.8322	0.37517
Residuals	16	5.4030e+13	3.3769e+12		

1469 ---
1470 Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1471 -----

1472 *EMMeans pairwise contrasts by treatment ("Treatment"):*

contrast	Treatment	estimate	SE	df	t.ratio	p.value
EV - irTOC1 W+OS		-2807381	1162217	16	-2.416	0.0280 *
EV - irTOC1 W+OS + MCP		-1307948	1162217	16	-1.125	0.2770

1473

1474 **Caffeoylputrescine:**

1475 ANOVA results for caffeoylputrescine concentration ("value") as
1476 response variable:

1477 Response: value

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	1	3.5858e+12	3.5858e+12	13.1777	0.0008734 ***
Treatment	1	4.3739e+12	4.3739e+12	16.0742	0.0002934 ***
Genotype:Treatment	1	2.0474e+11	2.0474e+11	0.7524	0.3914581
Residuals	36	9.7959e+12	2.7211e+11		

```

1491 ---
1492 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1493 -----
1494 EMMeans pairwise contrasts by treatment ("Treatment"):
1495 contrast Treatment estimate SE df t.ratio p.value
1496 EV - irTOC1 W+OS 455726 233285 36 1.954 0.0586 .
1497 EV - irTOC1 W+OS + MCP 741900 233285 36 3.180 0.0030 **
1498
1499 Dicaffeoylspermidine:
1500 ANOVA results for dicaffeoylspermidine concentration ("value") as
1501 response variable:
1502 Response: value
1503
1504 Df Sum Sq Mean Sq F value Pr(>F)
1505 Genotype 1 3.4405e+13 3.4405e+13 24.6086 1.694e-05 ***
1506 Treatment 1 2.0811e+12 2.0811e+12 1.4886 0.23037
1507 Genotype:Treatment 1 8.6750e+12 8.6750e+12 6.2049 0.01749 *
1508 Residuals 36 5.0331e+13 1.3981e+12
1509 ---
1510 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1511 -----
1512 EMMeans pairwise contrasts by treatment ("Treatment"):
1513 contrast Treatment estimate SE df t.ratio p.value
1514 EV - irTOC1 W+OS 923458 528788 36 1.746 0.0893 .
1515 EV - irTOC1 W+OS + MCP 2786248 528788 36 5.269 <.0001 ***
1516
1517 Secondary metabolites (roots):
1518 Nicotine:
1519 ANOVA results for nicotine concentration ("value") as response
1520 variable:
1521 Response: value
1522
1523 Df Sum Sq Mean Sq F value Pr(>F)
1524 Genotype 1 2.2261e+13 2.2261e+13 13.2554 0.002201 **
1525 Treatment 1 1.3004e+13 1.3004e+13 7.7435 0.013307 *
1526 Genotype:Treatment 1 2.6368e+07 2.6368e+07 0.0000 0.996887
1527 Residuals 16 2.6870e+13 1.6794e+12
1528 ---
1529 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1530 -----
1531 EMMeans pairwise contrasts by treatment ("Treatment"):
1532 contrast Treatment estimate SE df t.ratio p.value
1533 EV - irTOC1 W+OS -2112308 819601 16 -2.577 0.0203 *
1534 EV - irTOC1 W+OS + MCP -2107715 819601 16 -2.572 0.0205 *
1535
1536 Caffeoylputrescine:
1537 ANOVA results for caffeoylputrescine concentration ("value") as
1538 response variable:
1539 Response: value

```

```

1538                               Df      Sum Sq   Mean Sq F value Pr(>F)
1539 Genotype                  1 3.9958e+09 3995812901  1.0884 0.3123
1540 Treatment                 1 7.2446e+09 7244554470  1.9733 0.1792
1541 Genotype:Treatment       1 4.3849e+09 4384898597  1.1944 0.2906
1542 Residuals                 16 5.8740e+10 3671279922
1543 -----
1544 EMMeans pairwise contrasts by treatment ("Treatment"):
1545 contrast Treatment estimate   SE df t.ratio p.value
1546 EV - irTOC1 W+OS          -57883 38321 16 -1.510  0.1504
1547 EV - irTOC1 W+OS + MCP    1344 38321 16  0.035  0.9724
1548
1549 Dicaffeoylspermidine:
1550 ANOVA results for dicaffeoylspermidine concentration ("value") as
1551 response variable:
1552 Response: value
1553                               Df      Sum Sq   Mean Sq F value Pr(>F)
1554 Genotype                  1 2.4105e+08 241048267  0.3080 0.5866
1555 Treatment                 1 9.7492e+08 974923941  1.2456 0.2809
1556 Genotype:Treatment       1 4.6024e+08 460238585  0.5880 0.4543
1557 Residuals                 16 1.2523e+10 782665939
1558 -----
1559 EMMeans pairwise contrasts by treatment ("Treatment"):
1560 contrast Treatment estimate   SE df t.ratio p.value
1561 EV - irTOC1 W+OS          -16537 17694 16 -0.935  0.3639
1562 EV - irTOC1 W+OS + MCP    2651 17694 16  0.150  0.8828

```

1563 Figure 6:
 1564 Outlier removal:
 1565 One plant identified using our outlier procedure with divergent values
 1566 across a range of phenotypes was removed (EV control, replicate 1).
 1567 Overall lower replicate numbers, particularly for biomass and
 1568 secondary metabolite data and later time points of fitness correlate
 1569 data, were caused by an outbreak of a *Fusarium/Alternaria* pathosystem
 1570 infection during the second half of the field season.
 1571
 1572 Secondary metabolites:
Nicotine:
 1574 ANOVA results for nicotine concentration Peak area/g FW (Nicotine) as
 1575 response variable:
 1576 Response: Nicotine

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	2	6436.2	3218.1	3.8260	0.03612 *
Treatment	1	1.5	1.5	0.0018	0.96626
Genotype:Treatment	2	435.5	217.8	0.2589	0.77402
Residuals	24	20186.5	841.1		

1577 ---
 1583 Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
 1584 -----
 1585 Pairwise t-tests with pooled SD by Genotype (no *EMMeans* were
 1586 calculated due to lack of a significant interaction factor):
 1587
 1588 EE ET
 1589 ET 0.04 * -
 1590 TT 0.04 * 0.79
 1591
 1592 P value adjustment method: holm

Caffeoylputrescine:
 1595 ANOVA results for caffeoylputrescine concentration Peak area/g FW (CP)
 1596 as response variable:
 1597 Response: CP

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	2	1.2254	0.6127	1.1232	0.34110
Treatment	1	3.2132	3.2132	5.8901	0.02277 *
Genotype:Treatment	2	0.6040	0.3020	0.5536	0.58175
Residuals	25	13.6379	0.5455		

1598 ---
 1604 Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
 1605 -----
 1606 Pairwise t tests with pooled SD by Treatment (no *EMMeans* were
 1607 calculated due to lack of a significant interaction factor):
 1608

```
1609      Control
1610 W+OS 0.029 *
1611
1612 P value adjustment method: holm
1613
1614 Dicaffeoylspermidine:
1615 ANOVA results for dicaffeoylspermidine concentration Peak area/g FW
1616 (DCS) as response variable:
1617 Response: DCS
1618              Df     Sum Sq   Mean Sq F value Pr(>F)
1619 Genotype        2 6.0307e+11 3.0153e+11 3.9294 0.03282 *
1620 Treatment       1 1.1942e+10 1.1942e+10 0.1556 0.69656
1621 Genotype:Treatment 2 2.9374e+09 1.4687e+09 0.0191 0.98106
1622 Residuals      25 1.9184e+12 7.6737e+10
1623 ---
1624 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1625 -----
1626 Pairwise t tests with pooled SD by Genotype (no EMMeans were
1627 calculated due to lack of a significant interaction factor):
1628
1629    EE      ET
1630 ET 0.028 *  -
1631 TT 0.487  0.087 .
1632
1633 P value adjustment method: holm
```

```

1634 Reproductive correlates:
1635 Seed capsules
1636 ANOVA results for seed capsules (Capsules) as response variable:
1637 Response: Capsules
1638                               Df  Sum Sq Mean Sq F value Pr(>F)
1639 Genotype                   2   199.22  99.610 45.7673 < 2e-16 ***
1640 Treatment                  1     8.34   8.336  3.8299 0.05056 .
1641 Time                       19  1398.75  73.618 33.8250 < 2e-16 ***
1642 Genotype:Treatment         2    12.93   6.466  2.9708 0.05160 .
1643 Genotype:Time              38   409.37  10.773  4.9498 < 2e-16 ***
1644 Treatment:Time             19   67.80   3.568  1.6395 0.04051 *
1645 Genotype:Treatment:Time   38   55.07   1.449  0.6659 0.94133
1646 Residuals                 1320 2872.91   2.176
1647 ---
1648 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1649 -----
1650 EMMeans pairwise contrasts by genotype and time point in days
1651 ("Genotype", "Time"):
1652 Note: no seed capsules before day 30
1653 contrast      Genotype Time      estimate      SE      df t.ratio
1654 p.value
1655 Control - W+OS    EE       30        0.0000  0.616  1320  0.000
1656 1.0000
1657 Control - W+OS    ET       30        0.0769  0.591  1320  0.130
1658 0.8964
1659 Control - W+OS    TT       30        0.0000  0.602  1320  0.000
1660 1.0000
1661 Control - W+OS    EE       31       -0.3333  0.616  1320 -0.541
1662 0.5884
1663 Control - W+OS    ET       31       -0.0192  0.591  1320 -0.033
1664 0.9740
1665 Control - W+OS    TT       31        0.0000  0.602  1320  0.000
1666 1.0000
1667 Control - W+OS    EE       32       -0.0606  0.616  1320 -0.098
1668 0.9216
1669 Control - W+OS    ET       32        0.1282  0.591  1320  0.217
1670 0.8282
1671 Control - W+OS    TT       32        0.0000  0.602  1320  0.000
1672 1.0000
1673 Control - W+OS    EE       33       -0.1288  0.616  1320 -0.209
1674 0.8344
1675 Control - W+OS    ET       33        0.0833  0.591  1320  0.141
1676 0.8878
1677 Control - W+OS    TT       33        0.0000  0.602  1320  0.000
1678 1.0000
1679 Control - W+OS    EE       34       -0.1288  0.616  1320 -0.209
1680 0.8344

```

1681	Control - W+OS	ET	34	0.0833	0.591	1320	0.141
1682	0.8878						
1683	Control - W+OS	TT	34	0.0000	0.602	1320	0.000
1684	1.0000						
1685	Control - W+OS	EE	35	-0.1288	0.616	1320	-0.209
1686	0.8344						
1687	Control - W+OS	ET	35	0.0833	0.591	1320	0.141
1688	0.8878						
1689	Control - W+OS	TT	35	0.0000	0.602	1320	0.000
1690	1.0000						
1691	Control - W+OS	EE	36	-0.0909	0.616	1320	-0.148
1692	0.8827						
1693	Control - W+OS	ET	36	0.9295	0.591	1320	1.574
1694	0.1158						
1695	Control - W+OS	TT	36	0.0000	0.602	1320	0.000
1696	1.0000						
1697	Control - W+OS	EE	37	-0.0909	0.616	1320	-0.148
1698	0.8827						
1699	Control - W+OS	ET	37	0.9295	0.591	1320	1.574
1700	0.1158						
1701	Control - W+OS	TT	37	0.0000	0.602	1320	0.000
1702	1.0000						
1703	Control - W+OS	EE	38	-0.0909	0.616	1320	-0.148
1704	0.8827						
1705	Control - W+OS	ET	38	0.9295	0.591	1320	1.574
1706	0.1158						
1707	Control - W+OS	TT	38	0.0000	0.602	1320	0.000
1708	1.0000						
1709	Control - W+OS	EE	39	-0.0909	0.616	1320	-0.148
1710	0.8827						
1711	Control - W+OS	ET	39	0.9295	0.591	1320	1.574
1712	0.1158						
1713	Control - W+OS	TT	39	0.0000	0.602	1320	0.000
1714	1.0000						
1715	Control - W+OS	EE	40	1.7273	0.616	1320	2.805
1716	0.0051 **						
1717	Control - W+OS	ET	40	4.0833	0.591	1320	6.914
1718	<.0001 ***						
1719	Control - W+OS	TT	40	0.0000	0.602	1320	0.000
1720	1.0000						
1721	-----						

1722 EMMeans pairwise contrasts by treatment and time point in days
 1723 ("Treatment", "Time"):

1724 **Note:** no seed capsules before day 30

	contrast	Treat.	Time	estimate	SE	df	t.ratio	p.value
1726	EE - ET	Control	30	-0.0769	0.604	1320	-0.127	0.9911
1727	EE - TT	Control	30	0.0000	0.616	1320	0.000	1.0000
1728	ET - TT	Control	30	0.0769	0.591	1320	0.130	0.9907

1729	EE	-	ET	W+OS	30	0.0000	0.602	1320	0.000	1.0000
1730	EE	-	TT	W+OS	30	0.0000	0.602	1320	0.000	1.0000
1731	ET	-	TT	W+OS	30	0.0000	0.602	1320	0.000	1.0000
1732	EE	-	ET	Control	31	-0.2308	0.604	1320	-0.382	0.9228
1733	EE	-	TT	Control	31	0.0000	0.616	1320	0.000	1.0000
1734	ET	-	TT	Control	31	0.2308	0.591	1320	0.391	0.9193
1735	EE	-	ET	W+OS	31	0.0833	0.602	1320	0.138	0.9895
1736	EE	-	TT	W+OS	31	0.3333	0.602	1320	0.553	0.8447
1737	ET	-	TT	W+OS	31	0.2500	0.602	1320	0.415	0.9094
1738	EE	-	ET	Control	32	-0.1888	0.604	1320	-0.312	0.9476
1739	EE	-	TT	Control	32	0.2727	0.616	1320	0.443	0.8976
1740	ET	-	TT	Control	32	0.4615	0.591	1320	0.781	0.7145
1741	EE	-	ET	W+OS	32	0.0000	0.602	1320	0.000	1.0000
1742	EE	-	TT	W+OS	32	0.3333	0.602	1320	0.553	0.8447
1743	ET	-	TT	W+OS	32	0.3333	0.602	1320	0.553	0.8447
1744	EE	-	ET	Control	33	-0.5455	0.604	1320	-0.902	0.6388
1745	EE	-	TT	Control	33	0.4545	0.616	1320	0.738	0.7408
1746	ET	-	TT	Control	33	1.0000	0.591	1320	1.693	0.2081
1747	EE	-	ET	W+OS	33	-0.3333	0.602	1320	-0.553	0.8447
1748	EE	-	TT	W+OS	33	0.5833	0.602	1320	0.969	0.5969
1749	ET	-	TT	W+OS	33	0.9167	0.602	1320	1.522	0.2809
1750	EE	-	ET	Control	34	-0.5455	0.604	1320	-0.902	0.6388
1751	EE	-	TT	Control	34	0.4545	0.616	1320	0.738	0.7408
1752	ET	-	TT	Control	34	1.0000	0.591	1320	1.693	0.2081
1753	EE	-	ET	W+OS	34	-0.3333	0.602	1320	-0.553	0.8447
1754	EE	-	TT	W+OS	34	0.5833	0.602	1320	0.969	0.5969
1755	ET	-	TT	W+OS	34	0.9167	0.602	1320	1.522	0.2809
1756	EE	-	ET	Control	35	-0.5455	0.604	1320	-0.902	0.6388
1757	EE	-	TT	Control	35	0.4545	0.616	1320	0.738	0.7408
1758	ET	-	TT	Control	35	1.0000	0.591	1320	1.693	0.2081
1759	EE	-	ET	W+OS	35	-0.3333	0.602	1320	-0.553	0.8447
1760	EE	-	TT	W+OS	35	0.5833	0.602	1320	0.969	0.5969
1761	ET	-	TT	W+OS	35	0.9167	0.602	1320	1.522	0.2809
1762	EE	-	ET	Control	36	-0.9371	0.604	1320	-1.550	0.2678
1763	EE	-	TT	Control	36	1.8258	0.616	1320	2.965	0.0087 **
1764	ET	-	TT	Control	36	2.7628	0.591	1320	4.678	<.0001 ***
1765	EE	-	ET	W+OS	36	0.0833	0.602	1320	0.138	0.9895
1766	EE	-	TT	W+OS	36	1.9167	0.602	1320	3.182	0.0043 **
1767	ET	-	TT	W+OS	36	1.8333	0.602	1320	3.044	0.0067 **
1768	EE	-	ET	Control	37	-0.9371	0.604	1320	-1.550	0.2678
1769	EE	-	TT	Control	37	1.8258	0.616	1320	2.965	0.0087 **
1770	ET	-	TT	Control	37	2.7628	0.591	1320	4.678	<.0001 ***
1771	EE	-	ET	W+OS	37	0.0833	0.602	1320	0.138	0.9895
1772	EE	-	TT	W+OS	37	1.9167	0.602	1320	3.182	0.0043 **
1773	ET	-	TT	W+OS	37	1.8333	0.602	1320	3.044	0.0067 **
1774	EE	-	ET	Control	38	-0.9371	0.604	1320	-1.550	0.2678
1775	EE	-	TT	Control	38	1.8258	0.616	1320	2.965	0.0087 **
1776	ET	-	TT	Control	38	2.7628	0.591	1320	4.678	<.0001 ***

```

1777  EE - ET  W+OS   38      0.0833  0.602  1320  0.138  0.9895
1778  EE - TT  W+OS   38      1.9167  0.602  1320  3.182  0.0043  **
1779  ET - TT  W+OS   38      1.8333  0.602  1320  3.044  0.0067  **
1780  EE - ET  Control 39     -0.9371  0.604  1320  -1.550  0.2678
1781  EE - TT  Control 39     1.8258  0.616  1320  2.965  0.0087  **
1782  ET - TT  Control 39     2.7628  0.591  1320  4.678  <.0001  ***
1783  EE - ET  W+OS   39      0.0833  0.602  1320  0.138  0.9895
1784  EE - TT  W+OS   39      1.9167  0.602  1320  3.182  0.0043  **
1785  ET - TT  W+OS   39      1.8333  0.602  1320  3.044  0.0067  **
1786  EE - ET  Control 40    -2.2727  0.604  1320  -3.760  0.0005  ***
1787  EE - TT  Control 40    4.3106  0.616  1320  7.000  <.0001  ***
1788  ET - TT  Control 40    6.5833  0.591  1320  11.147 <.0001  ***
1789  EE - ET  W+OS   40      0.0833  0.602  1320  0.138  0.9895
1790  EE - TT  W+OS   40      2.5833  0.602  1320  4.289  0.0001  ***
1791  ET - TT  W+OS   40      2.5000  0.602  1320  4.151  0.0001  ***
1792
1793 P value adjustment: tukey method for comparing a family of 3 estimates
1794 -----
1795 EM Trends pairwise contrasts with seed capsules against time in days
1796 ("Time.trend"):
1797 $mtrends
1798   Treat. Genotype Time.trend      SE   df lower.CL upper.CL
1799   Control  EE        0.1700 0.0187 1428   0.1332   0.207
1800   W+OS    EE        0.1516 0.0179 1428   0.1164   0.187
1801   Control  ET        0.2511 0.0172 1428   0.2172   0.285
1802   W+OS    ET        0.1521 0.0179 1428   0.1169   0.187
1803   Control  TT        0.0237 0.0179 1428  -0.0115   0.059 .
1804   W+OS    TT        0.0237 0.0179 1428  -0.0115   0.059 .
1805
1806 Confidence level used: 0.95
1807
1808 $contrasts
1809   contrast          estimate      SE   df t.ratio p.value
1810  Control,EE - W+OS ,EE  0.018427 0.0260 1428   0.710  0.9808
1811  Control,EE - Control,ET -0.081077 0.0255 1428  -3.183  0.0186 *
1812  Control,EE - W+OS ,ET  0.017926 0.0260 1428   0.691  0.9830
1813  Control,EE - Control,TT 0.146246 0.0260 1428   5.635 <.0001  ***
1814  Control,EE - W+OS ,TT  0.146246 0.0260 1428   5.635 <.0001  ***
1815  W+OS ,EE - Control,ET -0.099504 0.0249 1428  -3.998  0.0009  ***
1816  W+OS ,EE - W+OS ,ET -0.000501 0.0254 1428  -0.020  1.0000
1817  W+OS ,EE - Control,TT 0.127820 0.0254 1428   5.036 <.0001  ***
1818  W+OS ,EE - W+OS ,TT  0.127820 0.0254 1428   5.036 <.0001  ***
1819  Control,ET - W+OS ,ET 0.099002 0.0249 1428   3.978  0.0010  ***
1820  Control,ET - Control,TT 0.227323 0.0249 1428   9.133 <.0001  ***
1821  Control,ET - W+OS ,TT  0.227323 0.0249 1428   9.133 <.0001  ***
1822  W+OS ,ET - Control,TT 0.128321 0.0254 1428   5.056 <.0001  ***
1823  W+OS ,ET - W+OS ,TT  0.128321 0.0254 1428   5.056 <.0001  ***
1824  Control,TT - W+OS ,TT 0.000000 0.0254 1428   0.000  1.0000

```

```

1825
1826 P value adjustment: tukey method for comparing a family of 6 estimates
1827
1828
1829 Flowers
1830 ANOVA results for flowers (Flowers) as response variable:
1831 Response: Flowers
1832
1833   Df Sum Sq Mean Sq F value Pr(>F)
1834 Genotype          2   890.8   445.4  5.1315 0.006567 **
1835 Treatment         1   118.2   118.2  1.3617 0.244393
1836 Time              7 29722.4  4246.1 48.9176 < 2.2e-16 ***
1837 Genotype:Treatment 2    11.2     5.6  0.0647 0.937331
1838 Genotype:Time      14   2255.7   161.1  1.8562 0.031829 *
1839 Treatment:Time     7   274.1    39.2  0.4511 0.868902
1840 Genotype:Treatment:Time 13   637.1    49.0  0.5646 0.880703
1841 Residuals          242 21005.6   86.8
1842 ---
1843 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1844 -----
1845 EMMeans pairwise contrasts by genotype and time point in days
("Genotype", "Time"):
1846 contrast       Genotype Timepoint estimate    SE  df t.ratio
1847 p.value
1848 Control - W+OS    EE      21      -0.3333  8.50 242 -0.039
1849 0.9688
1850 Control - W+OS    ET      21      -0.1667  7.12 242 -0.023
1851 0.9813
1852 Control - W+OS    TT      21      0.0000  8.50 242  0.000
1853 1.0000
1854 Control - W+OS    EE      24      -1.3333  7.61 242 -0.175
1855 0.8610
1856 Control - W+OS    ET      24      0.0000  7.61 242  0.000
1857 1.0000
1858 Control - W+OS    TT      24      0.0000  7.61 242  0.000
1859 1.0000
1860 Control - W+OS    EE      25      -1.2000  4.17 242 -0.288
1861 0.7736
1862 Control - W+OS    ET      25      0.2756  3.73 242  0.074
1863 0.9411
1864 Control - W+OS    TT      25      0.0909  3.89 242  0.023
1865 0.9814
1866 Control - W+OS    EE      28      -1.1444  4.28 242 -0.267
1867 0.7894
1868 Control - W+OS    ET      28      0.4336  3.82 242  0.114
1869 0.9097
1870 Control - W+OS    TT      28      -0.1818  3.97 242 -0.046
1871 0.9635

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```

1872 Control - W+OS    EE      31       -0.9028  4.53 242 -0.199
1873 0.8421
1874 Control - W+OS    ET      31       -0.1111  4.70 242 -0.024
1875 0.9811
1876 Control - W+OS    TT      31       -0.8611  4.53 242 -0.190
1877 0.8493
1878 Control - W+OS    EE      35       nonEst   NA   NA   NA
1879 NA (note: due to human error, only one EE plant was measured on day
1880 35)
1881 Control - W+OS    ET      35       10.0000  8.50 242  1.176
1882 0.2408
1883 Control - W+OS    TT      35       -15.3333 10.76 242 -1.425
1884 0.1554
1885 Control - W+OS    EE      36       0.2619   5.18 242  0.051
1886 0.9597
1887 Control - W+OS    ET      36       -0.8333  5.60 242 -0.149
1888 0.8818
1889 Control - W+OS    TT      36       5.2540   4.70 242  1.119
1890 0.2642
1891 Control - W+OS    EE      40       15.6667  7.12 242  2.202
1892 0.0286 *
1893 Control - W+OS    ET      40       9.7500  10.42 242  0.936
1894 0.3502
1895 Control - W+OS    TT      40       -1.8333  7.12 242 -0.258
1896 0.7969
1897 -----
1898 EM Trends pairwise contrasts with flowers against time ("DCS.trend"):
1899 $emtrends
1900 Treat. Genotype Time.trend    SE  df lower.CL upper.CL
1901 Control EE          2.37 0.310 277   1.763   2.98
1902 W+OS   EE          1.58 0.317 277   0.960   2.21
1903 Control ET          1.83 0.272 277   1.296   2.37
1904 W+OS   ET          1.57 0.359 277   0.861   2.27
1905 Control TT          1.35 0.303 277   0.750   1.94
1906 W+OS   TT          1.18 0.289 277   0.615   1.75
1907
1908 Confidence level used: 0.95
1909
1910 $contrasts
1911 contrast           estimate   SE  df t.ratio p.value
1912 Control,EE - W+OS ,EE     0.7884 0.443 277  1.778  0.4816
1913 Control,EE - Control,ET  0.5410 0.412 277  1.312  0.7784
1914 Control,EE - W+OS ,ET     0.8055 0.474 277  1.700  0.5333
1915 Control,EE - Control,TT  1.0255 0.434 277  2.365  0.1723
1916 Control,EE - W+OS ,TT     1.1891 0.424 277  2.807  0.0593 .
1917 W+OS   ,EE - Control,ET -0.2475 0.418 277 -0.592  0.9915
1918 W+OS   ,EE - W+OS ,ET     0.0171 0.479 277  0.036  1.0000
1919 W+OS   ,EE - Control,TT  0.2371 0.439 277  0.540  0.9945

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1920 W+OS ,EE - W+OS ,TT 0.4007 0.429 277 0.934 0.9375
1921 Control,ET - W+OS ,ET 0.2646 0.450 277 0.587 0.9918
1922 Control,ET - Control,TT 0.4846 0.408 277 1.189 0.8421
1923 Control,ET - W+OS ,TT 0.6482 0.397 277 1.632 0.5778
1924 W+OS ,ET - Control,TT 0.2200 0.470 277 0.468 0.9972
1925 W+OS ,ET - W+OS ,TT 0.3836 0.461 277 0.833 0.9612
1926 Control,TT - W+OS ,TT 0.1636 0.419 277 0.390 0.9988
1927
1928 P value adjustment: tukey method for comparing a family of 6 estimates
1929
1930 Biomass:
1931 Shoots
1932 ANOVA results for shoot biomass in grams (Shoot) as response variable:
1933 Response: Shoot
1934
1935 Genotype Df Sum Sq Mean Sq F value Pr(>F)
1936 Treat. 1 3.41 3.406 0.0607 0.80681
1937 Genotype:Treat. 2 539.99 269.995 4.8124 0.01424 *
1938 Residuals 35 1963.62 56.103
1939 ---
1940 Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1941 -----
1942 EMMeans pairwise contrasts by treatment ("Treat"):
1943 contrast Treat estimate SE df t.ratio p.value
1944 EE - ET Control -1.52 4.69 35 -0.324 0.9439
1945 EE - TT Control -12.26 4.00 35 -3.063 0.0114 *
1946 ET - TT Control -10.74 4.69 35 -2.288 0.0708 .
1947 EE - ET W+OS -3.51 4.93 35 -0.712 0.7582
1948 EE - TT W+OS 2.04 3.35 35 0.610 0.8155
1949 ET - TT W+OS 5.55 4.93 35 1.126 0.5045
1950
1951 P value adjustment: tukey method for comparing a family of 3 estimates
1952
1953 Roots
1954 ANOVA results for root biomass in grams (Root) as response variable:
1955 Response: Root
1956
1957 Genotype Df Sum Sq Mean Sq F value Pr(>F)
1958 Treat. 1 0.2854 0.2854 0.5208 0.475287
1959 Genotype:Treat. 2 8.6037 4.3019 7.8503 0.001526 **
1960 Residuals 35 19.1795 0.5480
1961 ---
1962 Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
1963 -----
1964 EMMeans pairwise contrasts by treatment ("Treat"):
1965 contrast Treat estimate SE df t.ratio p.value

```

```

1966  EE - ET  Control  -0.0886 0.464 35 -0.191  0.9801
1967  EE - TT  Control  -1.2921 0.396 35 -3.266  0.0067 ** 
1968  ET - TT  Control  -1.2035 0.464 35 -2.594  0.0358 *
1969  EE - ET  W+OS   -0.4419 0.487 35 -0.907  0.6396
1970  EE - TT  W+OS   0.4754 0.331 35  1.436  0.3339
1971  ET - TT  W+OS   0.9173 0.487 35  1.882  0.1588
1972
1973 P value adjustment: tukey method for comparing a family of 3 estimates

```

1974 Figure S2:

1975 Outlier removal:

1976 One outlier (266/212, W+W replicate 5) was identified by our outlier
 1977 procedure. This sample had an ethylene peak ~3x greater than the rest
 1978 of its cohort (genotype and treatment), but no other identifiable
 1979 phenotypic differences in the sampled plant. It was not possible to
 1980 re-analyze the original ethylene detector peak, but from experience,
 1981 this was treated as likely instrument error, and the outlier was
 1982 removed from the analysis.

1983 Ethylene burst:

1984 ANOVA results for ethylene abundance in nL/gFW ("Ethylene") as
 1985 response variable:

1986 Response: Ethylene

	Df	Sum Sq	Mean Sq	F value	Pr(>F)						
Genotype	4	282.1	70.5	1.3821	0.25387						
Treatment	1	3768.3	3768.3	73.8428	2.401e-11 ***						
Genotype:Treatment	4	608.8	152.2	2.9825	0.02784 *						
Residuals	49	2500.5	51.0								

Signif. codes:	0	'***'	0.001	'**'	0.01	'*'	0.05	'.'	0.1	' '	1

1994 -----

1995 EMMeans pairwise contrasts by Treatment ("Treatment"):

contrast	Treatment	estimate	SE	df	t.ratio	p.value
266/266 - 266/205	W+W	3.3278	4.12	49	0.807	0.9273
266/266 - 266/212	W+W	1.3150	4.33	49	0.304	0.9981
266/266 - 205/205	W+W	2.3030	4.12	49	0.558	0.9804
266/266 - 212/212	W+W	2.0635	4.12	49	0.500	0.9870
266/205 - 266/212	W+W	-2.0128	4.33	49	-0.465	0.9901
266/205 - 205/205	W+W	-1.0248	4.12	49	-0.248	0.9991
266/205 - 212/212	W+W	-1.2643	4.12	49	-0.307	0.9980
266/212 - 205/205	W+W	0.9880	4.33	49	0.228	0.9994
266/212 - 212/212	W+W	0.7485	4.33	49	0.173	0.9998
205/205 - 212/212	W+W	-0.2395	4.12	49	-0.058	1.0000
266/266 - 266/205	W+Ms	-15.9160	4.12	49	-3.859	0.0029 **
266/266 - 266/212	W+Ms	-3.8559	4.12	49	-0.935	0.8819
266/266 - 205/205	W+Ms	-6.5086	4.12	49	-1.578	0.5182
266/266 - 212/212	W+Ms	-3.7746	4.12	49	-0.915	0.8897

2011	266/205 - 266/212 W+Ms	12.0601	4.12	49	2.924	0.0398	*
2012	266/205 - 205/205 W+Ms	9.4074	4.12	49	2.281	0.1685	
2013	266/205 - 212/212 W+Ms	12.1414	4.12	49	2.944	0.0379	*
2014	266/212 - 205/205 W+Ms	-2.6527	4.12	49	-0.643	0.9671	
2015	266/212 - 212/212 W+Ms	0.0813	4.12	49	0.020	1.0000	
2016	205/205 - 212/212 W+Ms	2.7340	4.12	49	0.663	0.9633	
2017							
2018	P value adjustment: tukey method for comparing a family of 5 estimates						
2019							

2020 Figure S3:
 2021 Outlier removal:
 2022 No outliers were removed from this dataset.
 2023 Transcript abundance:
2024 ACS3:
 2025 ANOVA results for transcript abundance ("Normalized") as response
 2026 variable:
 2027 Response: Normalized

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	0.52455	0.52455	228.5444	4.197e-13 ***
Genotype	1	0.00015	0.00015	0.0670	0.7981
Treatment:Genotype	1	0.00018	0.00018	0.0783	0.7822
Residuals	22	0.05049	0.00230		

2028 ---
 2029 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
 2030 -----
 2031 Pairwise t tests with pooled SD by Treatment (no *EMMeans* were
 2032 calculated due to lack of a significant interaction factor):
 2033
 2034 Control
 2035 W+OS 3.8e-14 ***
 2036 P value adjustment method: holm
 2037
2038 ACO:
 2039 ANOVA results for transcript abundance ("Normalized") as response
 2040 variable:
 2041 Response: Normalized

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	2.09199	2.09199	731.4895	<2e-16 ***
Genotype	1	0.00000	0.00000	0.0000	0.9946
Treatment:Genotype	1	0.00037	0.00037	0.1281	0.7238
Residuals	22	0.06292	0.00286		

2042 ---
 2043 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
 2044 -----
 2045 Pairwise t tests with pooled SD by Treatment (no *EMMeans* were
 2046 calculated due to lack of a significant interaction factor):
 2047
 2048 Control
 2049 W+OS <2e-16 ***
 2050 P value adjustment method: holm
 2051
2052 ETR1:

```

2065 ANOVA results for transcript abundance ("Normalized") as response
2066 variable:
2067 Response: Normalized
2068          Df  Sum Sq  Mean Sq F value    Pr(>F)
2069 Treatment      1 0.058560 0.058560  8.3205 0.008605 ** 
2070 Genotype       1 0.006604 0.006604  0.9383 0.343234
2071 Treatment:Genotype 1 0.005808 0.005808  0.8253 0.373481
2072 Residuals     22 0.154837 0.007038
2073 ---
2074 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
2075 -----
2076 Pairwise t tests with pooled SD by Treatment (no EMMeans were
2077 calculated due to lack of a significant interaction factor):
2078
2079          Control
2080 W+OS      0.0079 **
2081
2082 P value adjustment method: holm
2083
2084 CTR1:
2085 ANOVA results for transcript abundance ("Normalized") as response
2086 variable:
2087 Response: Normalized
2088          Df  Sum Sq  Mean Sq F value    Pr(>F)
2089 Treatment      1 0.078012 0.078012 78.1985 1.076e-08 ***
2090 Genotype       1 0.000324 0.000324  0.3248   0.5745
2091 Treatment:Genotype 1 0.000497 0.000497  0.4980   0.4878
2092 Residuals     22 0.021947 0.000998
2093 ---
2094 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
2095 -----
2096 Pairwise t tests with pooled SD by Treatment (no EMMeans were
2097 calculated due to lack of a significant interaction factor):
2098
2099          Control
2100 W+OS      3.2e-09 ***
2101
2102 P value adjustment method: holm
2103
2104 EIN2:
2105 ANOVA results for transcript abundance ("Normalized") as response
2106 variable:
2107 Response: Normalized
2108          Df  Sum Sq  Mean Sq F value Pr(>F)
2109 Treatment      1 0.01717 0.017172  0.6242 0.4379
2110 Genotype       1 0.01742 0.017422  0.6333 0.4347
2111 Treatment:Genotype 1 0.03024 0.030241  1.0992 0.3058
2112 Residuals     22 0.60525 0.027511

```

```

2113
2114 EIN3:
2115 ANOVA results for transcript abundance ("Normalized") as response
2116 variable:
2117 Response: Normalized
2118             Df   Sum Sq   Mean Sq F value Pr(>F)
2119 Treatment       1 0.0059295 0.0059295  7.7812 0.01069 *
2120 Genotype        1 0.0006521 0.0006521  0.8558 0.36496
2121 Treatment:Genotype 1 0.0012192 0.0012192  1.5999 0.21915
2122 Residuals      22 0.0167647 0.0007620
2123 ---
2124 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
2125 -----
2126 Pairwise t tests with pooled SD by Treatment (no EMMeans were
2127 calculated due to lack of a significant interaction factor):
2128
2129          Control
2130 W+OS     0.011 *
2131
2132 P value adjustment method: holm
2133
2134 ERF189:
2135 ANOVA results for transcript abundance ("Normalized") as response
2136 variable:
2137 Response: Normalized
2138             Df   Sum Sq   Mean Sq F value Pr(>F)
2139 Treatment       1 1.7289e-09 1.7289e-09  9.9080 0.005872 **
2140 Genotype        1 2.8620e-11 2.8620e-11  0.1640 0.690553
2141 Treatment:Genotype 1 8.5890e-11 8.5890e-11  0.4923 0.492417
2142 Residuals      17 2.9664e-09 1.7449e-10
2143 ---
2144 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
2145 -----
2146 Pairwise t tests with pooled SD by Treatment (no EMMeans were
2147 calculated due to lack of a significant interaction factor):
2148
2149          Control
2150 W+OS     0.0041 **
2151
2152 P value adjustment method: holm
2153

```

2154 Figure S4:
 2155 Outlier removal:
 2156 4 seedlings (EV-0uM, 205-0uM, etr1-0uM, and EV-10uM) were removed from
 2157 the analysis because of delayed germination time.
 2158 Hypocotyl length:
 2159 ANOVA results for hypocotyl length ("Length") as response variable:
 2160 Response: Length
 2161

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	3	37.404	12.4679	85.2925	< 2.2e-16 ***
Treatment	3	7.289	2.4298	16.6221	4.274e-09 ***
Genotype:Treatment	9	6.503	0.7226	4.9431	1.197e-05 ***
Residuals	120	17.541	0.1462		

 2162 ---
 2163 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
 2164

EMMeans pairwise contrasts by Genotype ("Genotype"):						
contrast	Genotype	estimate	SE	df	t.ratio	p.value
0 - 0.2	EV	0.7167	0.247	120	2.904	0.0225
0 - 0.5	EV	0.7730	0.240	120	3.226	0.0087
0 - 10	EV	0.9443	0.247	120	3.826	0.0012
0.2 - 0.5	EV	0.0563	0.213	120	0.265	0.9935
0.2 - 10	EV	0.2275	0.221	120	1.031	0.7319
0.5 - 10	EV	0.1712	0.213	120	0.805	0.8520
0 - 0.2	irTOC1-205	0.3993	0.180	120	2.216	0.1249
0 - 0.5	irTOC1-205	0.7092	0.176	120	4.037	0.0005
0 - 10	irTOC1-205	0.8929	0.176	120	5.083	<.0001
0.2 - 0.5	irTOC1-205	0.3099	0.176	120	1.764	0.2958
0.2 - 10	irTOC1-205	0.4936	0.176	120	2.810	0.0292
0.5 - 10	irTOC1-205	0.1837	0.171	120	1.074	0.7058
0 - 0.2	irTOC1-212	0.4097	0.176	120	2.332	0.0965
0 - 0.5	irTOC1-212	0.6590	0.176	120	3.751	0.0015
0 - 10	irTOC1-212	1.1746	0.180	120	6.517	<.0001
0.2 - 0.5	irTOC1-212	0.2493	0.171	120	1.458	0.4661
0.2 - 10	irTOC1-212	0.7648	0.176	120	4.354	0.0002
0.5 - 10	irTOC1-212	0.5155	0.176	120	2.935	0.0206
0 - 0.2	35S-etr1	-0.1687	0.180	120	-0.936	0.7857
0 - 0.5	35S-etr1	-0.3638	0.180	120	-2.018	0.1870
0 - 10	35S-etr1	-0.1995	0.176	120	-1.136	0.6684
0.2 - 0.5	35S-etr1	-0.1951	0.180	120	-1.083	0.7008
0.2 - 10	35S-etr1	-0.0308	0.176	120	-0.175	0.9981
0.5 - 10	35S-etr1	0.1643	0.176	120	0.935	0.7860

 2165

P value adjustment: tukey method for comparing a family of 4 estimates

 2166

2167

2198 Figure S5:

2199 Outlier removal:

2200 No outliers were removed from the data utilized in panel a. For the
 2201 data in panel b, one plant identified using our outlier procedure with
 2202 divergent values across a range of phenotypes was removed (EV control,
 2203 replicate 1).

2204 Secondary metabolites:

2205 **Chlorogenic acid (a) :**

2206 ANOVA results for chlorogenic acid concentration Peak area/g FW
 2207 ("CGA") as response variable:

2208 Response: CGA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
as.factor(Time)	1	20.793	20.7932	10.3272	0.003127 **
Genotype	2	31.777	15.8886	7.8913	0.001763 **
as.factor(Time):Genotype	2	1.255	0.6277	0.3118	0.734503
Residuals	30	60.403	2.0134		

2209 ---
 2210 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
 2211 -----
 2212 *EMMeans* pairwise contrasts by time point in hours ("Time"):
 2213 contrast Time estimate SE df t.ratio p.value
 2214 EV - irTOC1 0 1.895 0.819 30 2.313 0.0692 .
 2215 EV - irZTL 0 2.423 0.819 30 2.958 0.0160 *
 2216 irTOC1 - irZTL 0 0.528 0.819 30 0.645 0.7968
 2217 EV - irTOC1 72 1.972 0.819 30 2.407 0.0568 .
 2218 EV - irZTL 72 1.672 0.819 30 2.041 0.1199
 2219 irTOC1 - irZTL 72 -0.300 0.819 30 -0.366 0.9290
 2220
 2221 P value adjustment: tukey method for comparing a family of 3 estimates
 2222
 2223 **Chlorogenic acid (b) :**
 2224 ANOVA results for transcript abundance ("") as response variable:
 2225 Response: CGA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	5.3998e+11	5.3998e+11	2.7241	0.111872
Genotype	2	2.2326e+12	1.1163e+12	5.6316	0.009878 **
Treatment:Genotype	2	1.6324e+11	8.1619e+10	0.4118	0.667078
Residuals	24	4.7573e+12	1.9822e+11		

2226 ---
 2227 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
 2228 -----
 2229 *EMMeans* pairwise contrasts by Treatment ("Treatment"):
 2230 contrast Treatment estimate SE df t.ratio p.value
 2231 EE - ET cc -595829 287389 24 -2.073 0.1168
 2232 EE - TT cc -71570 340044 24 -0.210 0.9759
 2233 ET - TT cc 524260 314819 24 1.665 0.2389

```
2244 EE - ET cm      -356258 269595 24 -1.321  0.3974
2245 EE - TT cm      315481 269595 24  1.170  0.4819
2246 ET - TT cm      671738 257049 24  2.613  0.0390 *
2247
2248 P value adjustment: tukey method for comparing a family of 3 estimates
2249
2250
2251
```