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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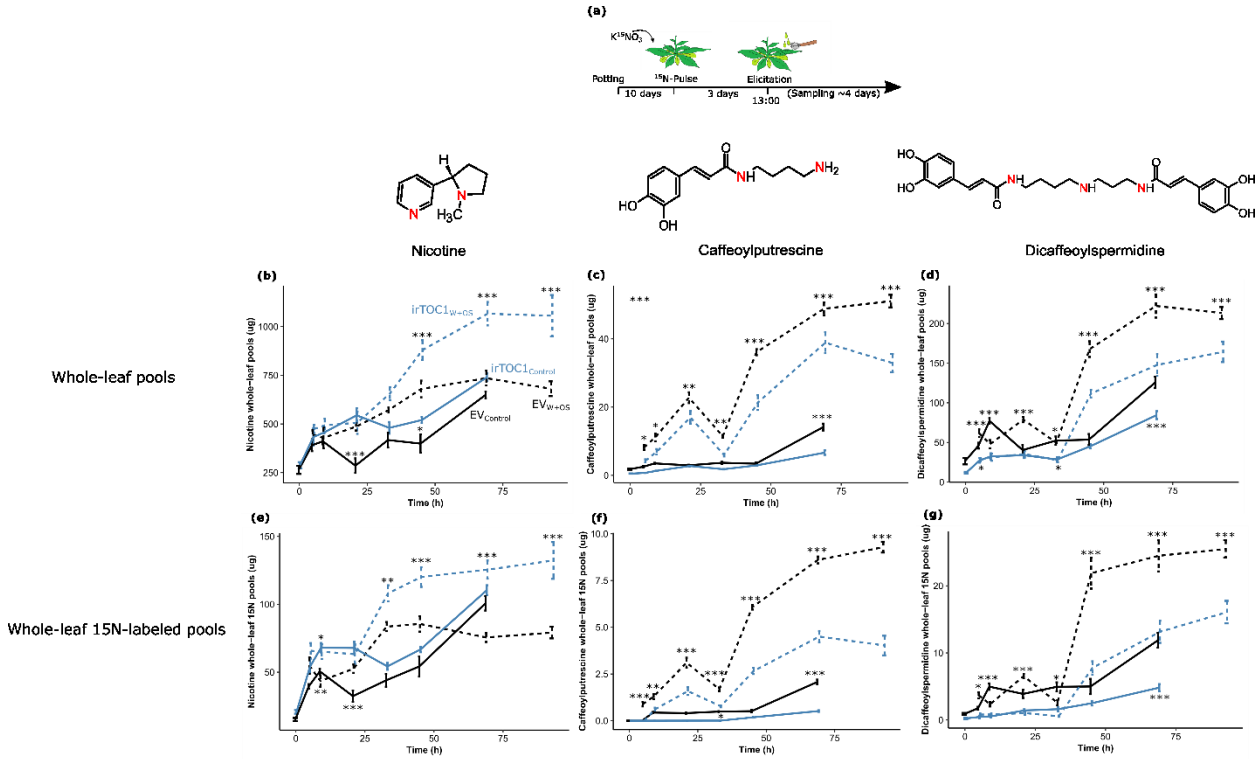



Figure S1

**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 15N-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.

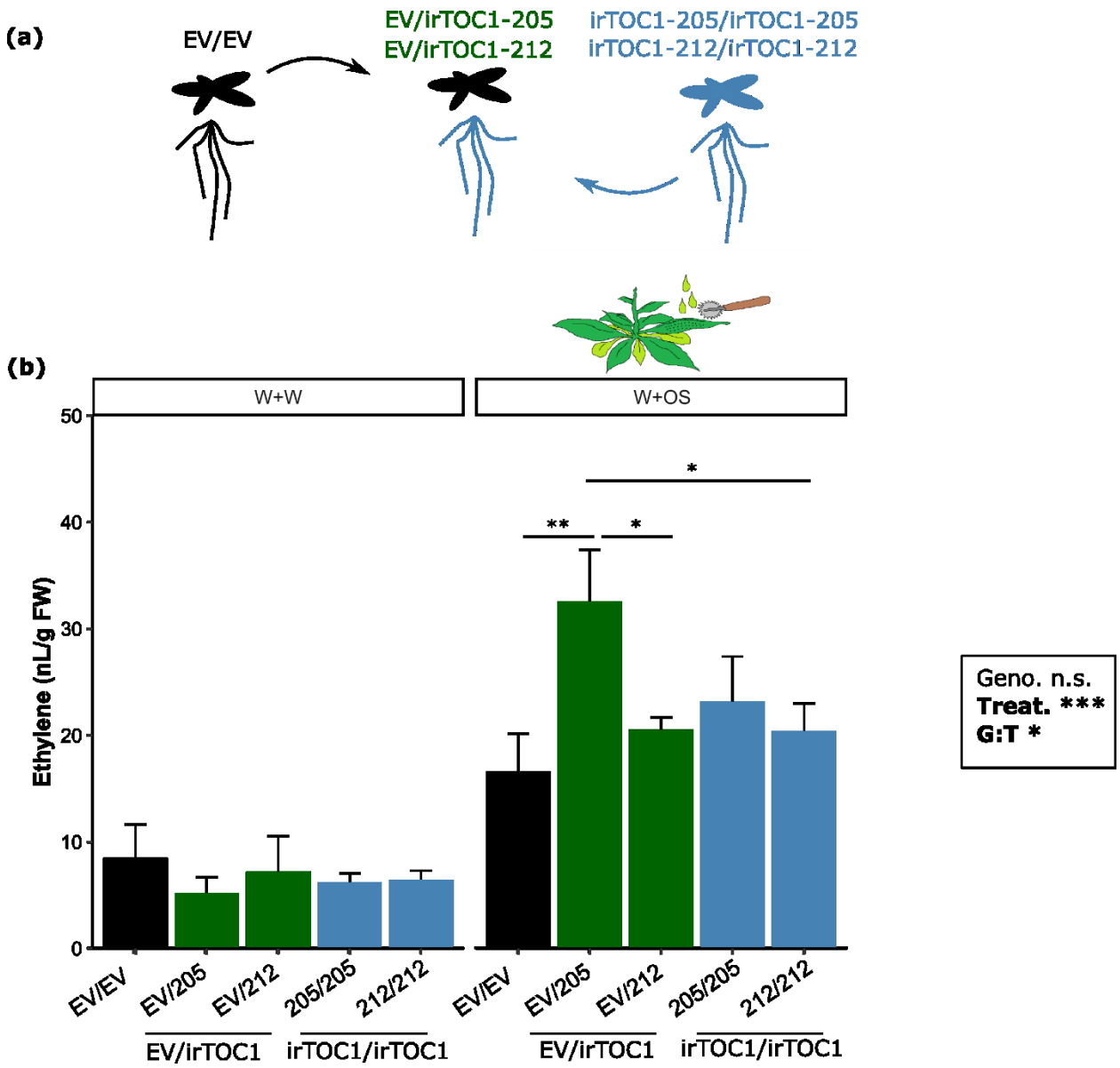



Figure S2

**Figure S2: Ethylene burst after elicitation with *Manduca sexta* oral secretions is not affected by whole-plant nor root-only *TOC1* silencing.** (a) Schematic of two independent silenced lines used for grafting and ethylene analysis in the glasshouse: EV/EV (black), EV/irTOC1 (green), and irTOC1/irTOC1 (blue) micrografted plants. (b) Ethylene levels 5h after elicitation in micrografted EV and *TOC1*-silenced lines. 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.

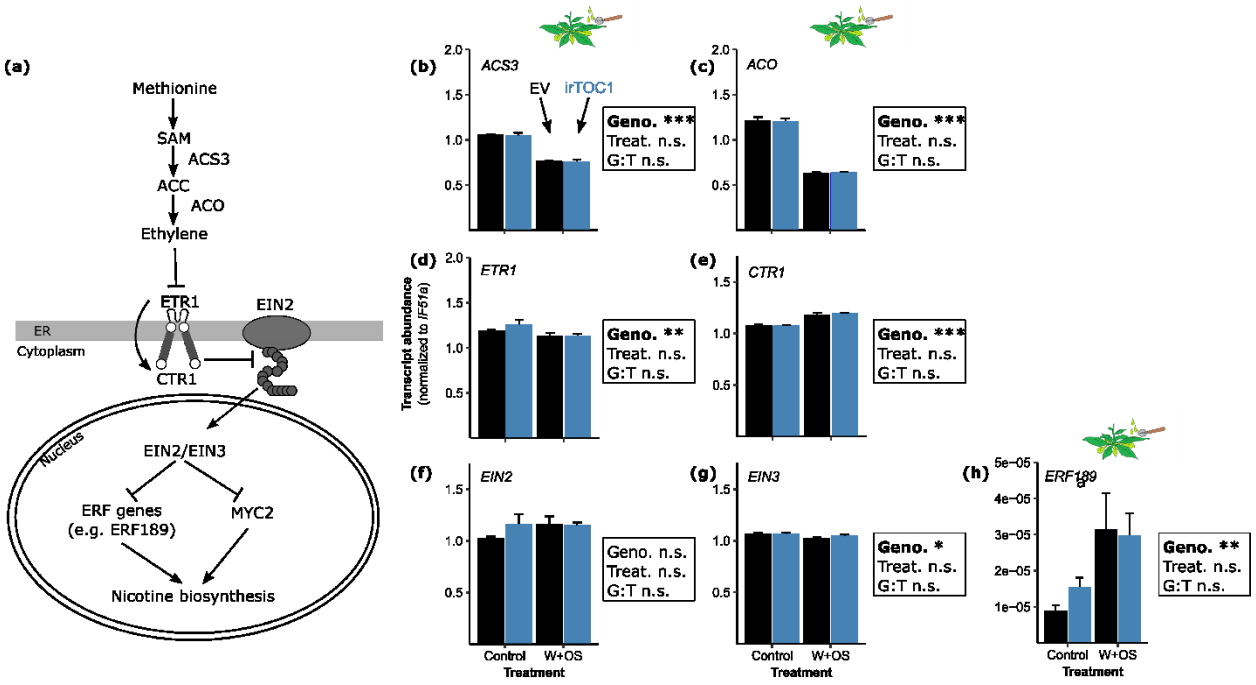
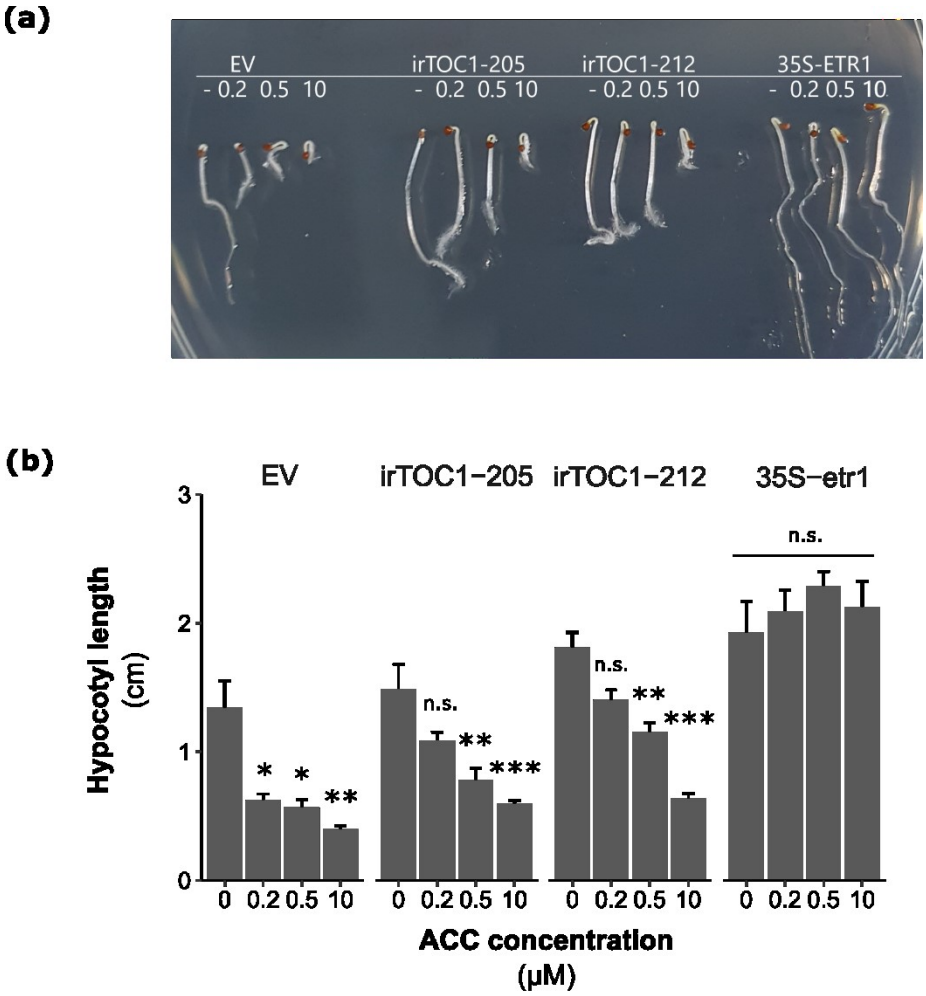



Figure S3

**Figure S3: Effect of *TOC1* silencing on transcript abundance of various ethylene receptors and downstream targets 1 hour after simulated herbivory.** (a) Schematic of ethylene biosynthesis and signaling. Ethylene biosynthesis (b,c), perception (d,e) and downstream signaling (f-g) are significantly affected by W+OS treatment, but not by TOC1 silencing 1h after elicitation. (h) Nicotine biosynthesis-targeting transcription factors, such as ERF189, are not abundant and affected by treatment but not genotype in leaf tissues. 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. SAM: *S*-adenosyl-L-methionine; ACC: 1-Aminocyclopropane-1-carboxylic acid; *ACS3: ACC SYNTHASE3; ACO: ACC OXIDASE; ETR1: ETHYLENE RESPONSE1; CTR1: CONSTITUTIVE RESPONSE1; EIN2: ETHYLENE INSENSITIVE2; EIN3: ETHYLENE INSENSITIVE3; ERF189: ETHYLENE RESPONSE FACTOR189*




# Figure S4

**Figure S4: Effect of silencing *NaTOC1* on ethylene triple response.** (a) Representative seedlings after 10 days under dark conditions exposed to ACC concentrations of 0, 0.2, 0.5, and 10 uM. (b) Hypocotyl lengths of EV, the ethylene-insensitive 35S-etr1 line, and two independent irTOC1 lines. N=4-7 for EV per treatment, 8-10 for other genotypes per treatment, 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, ns: not significant.

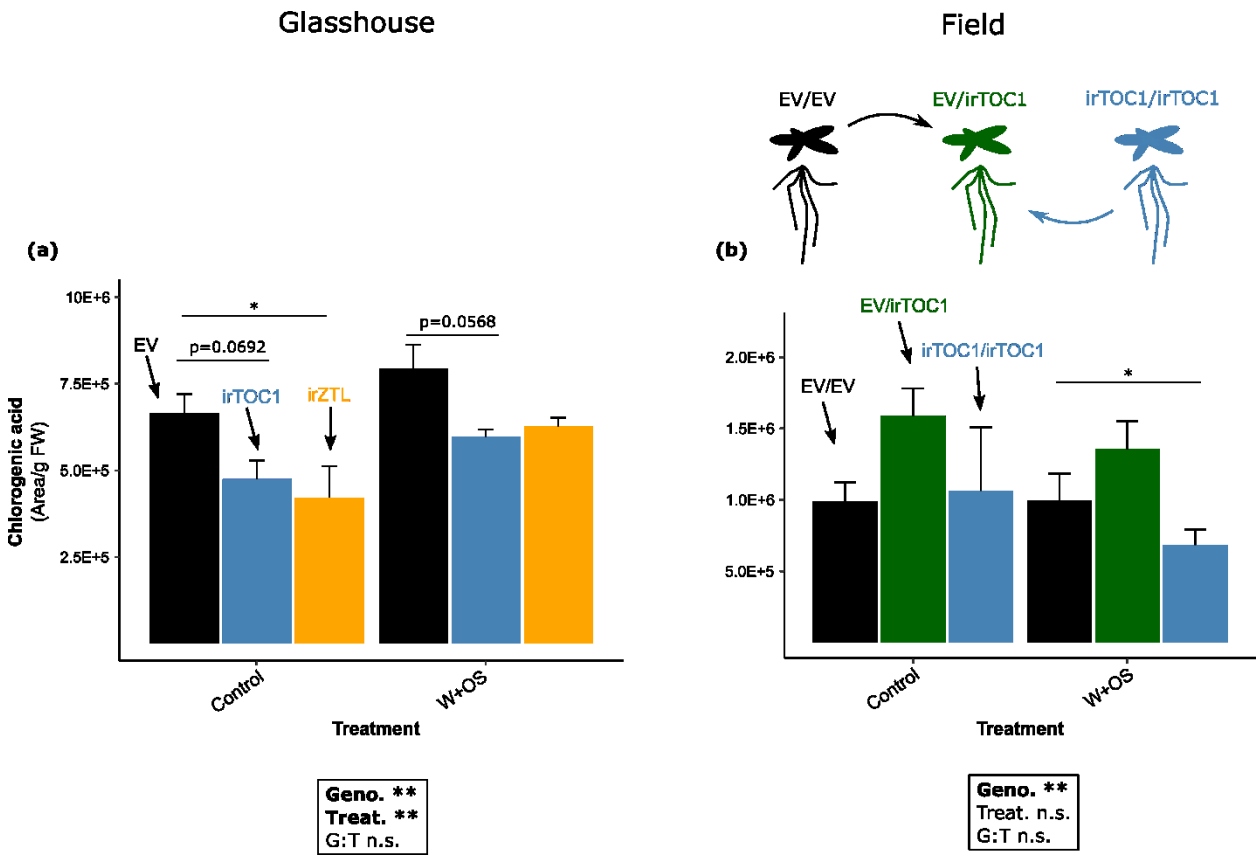



**Figure S5: Chlorogenic acid accumulation is attenuated in irTOC1 plants 72h after simulated herbivory.** (a) Levels of chlorogenic acid in EV, irTOC1, and irZTL plants under glasshouse conditions. (b) Schematic of two independent silenced lines used for grafting and ethylene analysis in the glasshouse: EV/EV (black), EV/irTOC1 (green), and irTOC1/irTOC1 (blue) micrografted plants, and levels of chlorogenic acid under field conditions in whole-plant and root-only *TOC1*-silenced plants. N=6 for (a), 4-6 for (b); 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.

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

| Gene | Forward | Reverse |
|---|---|---|
| **IF5a-2 (NIATv7_g63799)** | 5'-GTCGGACGAAGAACACCATT | 5'-CACATCACAGTTGTGGGAGG |
| **EF1a (NIATv7_g65928)** | 5'-CCACACTTCCCACATTGCTGTCA | 5'-CGCATGTCCCTCACAGCAAAAC |
| **NaLOX3 (NIATv7_g32174)** | 5'-GAATTTGAGAACAAGGAGAGTACTG | 5'- CTTAACAATTGTTTCCTTCAGATCTTCC |
| **NaAOC (NIATv7_g34489)** | 5'- AACTACCTAACCCTCTCATTTCTCA | 5'- AAGCGAAGATAGGCAGGGC |
| **NaOPR3 (NIATv7_g36098)** | 5'- AATGGAGTTGGAGTTTGTTT | 5'- AGGTGGTTGAAGCAGTCGTT |
| **NaTD (NIATv7_g00426)** | 5'-TAAGGCATTTGATGGGAGGC | 5'-TCTCCCTGTTCACGATAATGGAA |
| **NaMYC2 (NIATv7_g16429)** | 5'- GGCCCGGAACAACTACTACA | 5'- CCCCGTCGATTAAAGTCTGA |
| **NaCOI1 (NIATv7_g03444)** | 5'- CAGGGCATCTTCAGCTGGTC | 5'- CGGGATGCTCAGCAACGA |
| **NaMYB8 (NIATv7_g41919)** | 5'-TCCAAGTGAAAAAGCAAACTGAG | 5'-AGCTTCCCGTGCTCTCAATT |
| **NaACS3 (NIATv7_g57945)** | 5'- ATTCTCCTTTGGTCAGGA | 5'- ACTCCAAACTTACTTGTTTATTT |
| **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'-ACTGGCCTTTTATTGCTGACA | 5'-AGTGCTGTAATCTGATTGGAA |
| **NaEIN3 (NIATv7_ g04236)** | 5'-GGAGGAGGAAGAGATACCCTCA | 5'-TGCAGCCAACAAAGAGACTTA |
| **NaERF189 (NIATv7_g20472)** | 5'-TACTCCGAGTCCGAGGAAAA | 5'-GAAAGCGGCTTGATCGTAAG |

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## Notes S1: Expanded statistical information and outlier removal.

# Figure 1:

Outlier removal:

```
No outliers were removed from this dataset.
```

Transcript abundances:

**AOC:**

```
ANOVA results for transcript abundance (“expr”) as response variable:
Response: expr
                Df  Sum Sq Mean Sq F value    Pr(>F)
Time1            5 21.0451  4.2090 66.7750 < 2.2e-16 ***
Genotype         2  0.0458  0.0229  0.3634  0.697924
Time1:Genotype 10  2.0892  0.2089  3.3145  0.004063 **
Residuals       35  2.2061  0.0630
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
----------------------------------------------
EMMeans pairwise contrasts by time point (“Time1”):
contrast        Time1 estimate    SE df t.ratio p.value
 EV - irTOC1     0      0.07422 0.205 35  0.362  0.9304
 EV - irZTL      0      0.06926 0.205 35  0.338  0.9391
 irTOC1 - irZTL 0     -0.00497 0.205 35 -0.024  0.9997
 EV - irTOC1     30     0.36094 0.205 35  1.761  0.1976
 EV - irZTL      30    -0.44740 0.205 35 -2.183  0.0883 .
 irTOC1 - irZTL 30    -0.80835 0.205 35 -3.943  0.0010 **
 EV - irTOC1     60    -0.12410 0.229 35 -0.541  0.8515
 EV - irZTL      60    -0.36928 0.229 35 -1.611  0.2544
 irTOC1 - irZTL 60    -0.24518 0.205 35 -1.196  0.4634
 EV - irTOC1     90    -0.12317 0.205 35 -0.601  0.8206
 EV - irZTL      90    -0.25823 0.205 35 -1.260  0.4270
 irTOC1 - irZTL 90    -0.13506 0.205 35 -0.659  0.7886
 EV - irTOC1     120   -0.19916 0.205 35 -0.972  0.5994
 EV - irZTL      120    0.29654 0.205 35  1.447  0.3287
 irTOC1 - irZTL 120    0.49570 0.205 35  2.418  0.0533 .
 EV - irTOC1     180   -0.33021 0.205 35 -1.611  0.2545
 EV - irZTL      180    0.23639 0.205 35  1.153  0.4886
 irTOC1 - irZTL 180    0.56661 0.205 35  2.764  0.0240 *

P value adjustment: tukey method for comparing a family of 3 estimates
----------------------------------------------
EM Trends pairwise contrasts with transcript abundance against Time
(“Time”):
$emtrends
 Genotype Time.trend      SE df lower.CL upper.CL
 EV          0.00863 0.00184 47  0.00492  0.01233
```

```
 irTOC1      0.01164 0.00183 47  0.00795  0.01533
 irZTL       0.00589 0.00183 47  0.00220  0.00958

Confidence level used: 0.95

$contrasts
 contrast       estimate      SE df t.ratio p.value
 EV - irTOC1    -0.00302 0.00260 47 -1.161  0.4821
 EV - irZTL      0.00274 0.00260 47  1.054  0.5470
 irTOC1 - irZTL  0.00576 0.00259 47  2.219  0.0784 .

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

**COI1:**

```
ANOVA results for transcript abundance ("expr") as response variable:
Response: expr
               Df Sum Sq Mean Sq F value    Pr(>F)
Time1           5 37.759  7.5518 51.6868 1.167e-14 ***
Genotype        2  0.162  0.0808  0.5527   0.58063
Time1:Genotype 10  4.255  0.4255  2.9121   0.01002 *
Residuals      33  4.822  0.1461
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
EMMeans pairwise contrasts by time point ("Time1"):
contrast       Time1 estimate    SE df t.ratio p.value
 EV - irTOC1    0      0.50865 0.312 33  1.630  0.2476
 EV - irZTL     0      1.33528 0.441 33  3.025  0.0129 *
 irTOC1 - irZTL 0      0.82663 0.441 33  1.873  0.1625
 EV - irTOC1    30    -0.79094 0.312 33 -2.534  0.0418 *
 EV - irZTL     30     0.43901 0.312 33  1.407  0.3492
 irTOC1 - irZTL 30     1.22995 0.312 33  3.941  0.0011 **
 EV - irTOC1    60     0.39290 0.349 33  1.126  0.5052
 EV - irZTL     60     0.04688 0.349 33  0.134  0.9901
 irTOC1 - irZTL 60    -0.34603 0.312 33 -1.109  0.5156
 EV - irTOC1    90     0.28191 0.312 33  0.903  0.6421
 EV - irZTL     90     0.03168 0.312 33  0.101  0.9943
 irTOC1 - irZTL 90    -0.25023 0.312 33 -0.802  0.7046
 EV - irTOC1    120    0.00236 0.312 33  0.008  1.0000
 EV - irZTL     120   -0.10542 0.312 33 -0.338  0.9392
 irTOC1 - irZTL 120   -0.10777 0.312 33 -0.345  0.9365
 EV - irTOC1    180   -0.32190 0.312 33 -1.031  0.5627
 EV - irZTL     180   -0.40807 0.312 33 -1.308  0.4011
 irTOC1 - irZTL 180   -0.08617 0.312 33 -0.276  0.9589

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

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

```
$emtrends
 Genotype Time.trend      SE df lower.CL  upper.CL
 EV          -0.0147 0.00271 45  -0.0202 -0.009257
 irTOC1      -0.0127 0.00270 45  -0.0182 -0.007302
 irZTL       -0.0053 0.00308 45  -0.0115  0.000904

Confidence level used: 0.95

$contrasts
 contrast       estimate      SE df t.ratio p.value
 EV - irTOC1    -0.00197 0.00383 45 -0.515  0.8645
 EV - irZTL     -0.00942 0.00410 45 -2.297  0.0665 .
 irTOC1 - irZTL -0.00745 0.00410 45 -1.819  0.1751

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

**LOX3:**

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

```
Response: expr
               Df  Sum Sq Mean Sq F value    Pr(>F)
Time1           5 31.2535  6.2507 101.015 < 2.2e-16 ***
Genotype        2  3.5390  1.7695  28.596 3.670e-08 ***
Time1:Genotype 10  6.5647  0.6565  10.609 4.524e-08 ***
Residuals      36  2.2276  0.0619
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

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

```
contrast       Time1 estimate    SE df t.ratio p.value
 EV - irTOC1    0      0.03491 0.203 36  0.172  0.9839
 EV - irZTL     0      0.04726 0.203 36  0.233  0.9706
 irTOC1 - irZTL 0      0.01235 0.203 36  0.061  0.9980
 EV - irTOC1    30     0.71325 0.203 36  3.512  0.0034 **
 EV - irZTL     30    -1.24614 0.203 36 -6.135  <.0001 ***
 irTOC1 - irZTL 30    -1.95938 0.203 36 -9.647  <.0001 ***
 EV - irTOC1    60    -0.78493 0.203 36 -3.865  0.0013 **
 EV - irZTL     60    -0.66602 0.203 36 -3.279  0.0064 **
 irTOC1 - irZTL 60     0.11892 0.203 36  0.585  0.8287
 EV - irTOC1    90    -0.70667 0.203 36 -3.479  0.0037 **
 EV - irZTL     90    -1.40826 0.203 36 -6.934  <.0001 ***
 irTOC1 - irZTL 90    -0.70158 0.203 36 -3.454  0.0040 **
 EV - irTOC1    120    0.00818 0.203 36  0.040  0.9991
 EV - irZTL     120   -0.25737 0.203 36 -1.267  0.4226
 irTOC1 - irZTL 120   -0.26555 0.203 36 -1.307  0.4003
 EV - irTOC1    180   -0.09430 0.203 36 -0.464  0.8883
 EV - irZTL     180   -0.06243 0.203 36 -0.307  0.9493
```

```
 irTOC1 - irZTL 180    0.03187 0.203 36  0.157  0.9865

P value adjustment: tukey method for comparing a family of 3 estimates
----------------------------------------------
EM Trends pairwise contrasts with transcript abundance against Time
("Time"):
$emtrends
 Genotype Time.trend      SE df lower.CL upper.CL
 EV        -0.000717 0.00361 48 -0.00798  0.00655
 irTOC1     0.001137 0.00361 48 -0.00613  0.00840
 irZTL     -0.002680 0.00361 48 -0.00994  0.00458

Confidence level used: 0.95

$contrasts
 contrast       estimate      SE df t.ratio p.value
 EV - irTOC1    -0.00185 0.00511 48 -0.363  0.9301
 EV - irZTL      0.00196 0.00511 48  0.384  0.9220
 irTOC1 - irZTL  0.00382 0.00511 48  0.747  0.7369

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

**MYB8:**

```
ANOVA results for transcript abundance ("expr") as response variable:
Response: expr
               Df Sum Sq Mean Sq F value    Pr(>F)
Time1           5 60.234 12.0467 477.804 < 2.2e-16 ***
Genotype        2  5.908  2.9540 117.163 < 2.2e-16 ***
Time1:Genotype 10 21.935  2.1935  86.998 < 2.2e-16 ***
Residuals      36  0.908  0.0252
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
EMMeans pairwise contrasts by time point ("Time1"):
contrast       Time1 estimate   SE df t.ratio p.value
 EV - irTOC1    0       0.1384 0.13 36   1.067 0.5402
 EV - irZTL     0       0.0955 0.13 36   0.737 0.7435
 irTOC1 - irZTL 0      -0.0429 0.13 36  -0.331 0.9416
 EV - irTOC1    30      1.6501 0.13 36  12.728 <.0001 ***
 EV - irZTL     30     -0.8798 0.13 36  -6.786 <.0001 ***
 irTOC1 - irZTL 30     -2.5299 0.13 36 -19.514 <.0001 ***
 EV - irTOC1    60      2.9016 0.13 36  22.381 <.0001 ***
 EV - irZTL     60      2.9134 0.13 36  22.472 <.0001 ***
 irTOC1 - irZTL 60      0.0118 0.13 36   0.091 0.9955
 EV - irTOC1    90      0.6163 0.13 36   4.754 0.0001 ***
 EV - irZTL     90      0.4326 0.13 36   3.337 0.0055 **
 irTOC1 - irZTL 90     -0.1837 0.13 36  -1.417 0.3430
 EV - irTOC1    120    -0.2173 0.13 36  -1.676 0.2280
```

```
 EV - irZTL     120     0.2356 0.13 36   1.817 0.1785
 irTOC1 - irZTL 120     0.4529 0.13 36   3.493 0.0036 **
 EV - irTOC1    180    -0.2425 0.13 36  -1.870 0.1620
 EV - irZTL     180    -0.0475 0.13 36  -0.366 0.9289
 irTOC1 - irZTL 180     0.1950 0.13 36   1.504 0.3010

P value adjustment: tukey method for comparing a family of 3 estimates
---------------------------------------------
EM Trends pairwise contrasts with transcript abundance against Time
("Time"):
$emtrends
 Genotype Time.trend      SE df lower.CL upper.CL
 EV         -0.00674 0.00507 48 -0.01693  0.00345
 irTOC1      0.00175 0.00507 48 -0.00844  0.01194
 irZTL      -0.00613 0.00507 48 -0.01632  0.00406

Confidence level used: 0.95

$contrasts
 contrast        estimate      SE df t.ratio p.value
 EV - irTOC1    -0.008495 0.00717 48 -1.185  0.4676
 EV - irZTL     -0.000615 0.00717 48 -0.086  0.9959
 irTOC1 - irZTL  0.007879 0.00717 48  1.099  0.5191

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

**MYC2:**

```
ANOVA results for transcript abundance ("expr") as response variable:
Response: expr
               Df Sum Sq Mean Sq F value    Pr(>F)
Time1           5 33.608  6.7217 164.557 < 2.2e-16 ***
Genotype        2  2.272  1.1362  27.816 5.872e-08 ***
Time1:Genotype 10  5.995  0.5995  14.677 8.950e-10 ***
Residuals      35  1.430  0.0408
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
EMMeans pairwise contrasts by time point ("Time1"):
contrast       Time1 estimate    SE df t.ratio p.value
 EV - irTOC1    0      -0.0353 0.165 35 -0.214  0.9751
 EV - irZTL     0      -0.0712 0.165 35 -0.432  0.9027
 irTOC1 - irZTL 0      -0.0360 0.165 35 -0.218  0.9742
 EV - irTOC1    30      2.0008 0.165 35 12.125  <.0001 ***
 EV - irZTL     30      1.8269 0.165 35 11.071  <.0001 ***
 irTOC1 - irZTL 30     -0.1739 0.165 35 -1.054  0.5487
 EV - irTOC1    60      0.7174 0.184 35  3.888  0.0012 **
 EV - irZTL     60      0.5789 0.165 35  3.508  0.0035 **
 irTOC1 - irZTL 60     -0.1385 0.184 35 -0.751  0.7352
```

```
 EV - irTOC1    90      0.1437 0.165 35  0.871  0.6621
 EV - irZTL     90      0.0136 0.165 35  0.082  0.9963
 irTOC1 - irZTL 90     -0.1301 0.165 35 -0.788  0.7126
 EV - irTOC1    120    -0.0265 0.165 35 -0.160  0.9859
 EV - irZTL     120     0.0766 0.165 35  0.464  0.8884
 irTOC1 - irZTL 120     0.1030 0.165 35  0.624  0.8078
 EV - irTOC1    180    -0.0592 0.165 35 -0.359  0.9317
 EV - irZTL     180     0.1308 0.165 35  0.792  0.7101
 irTOC1 - irZTL 180     0.1899 0.165 35  1.151  0.4898

P value adjustment: tukey method for comparing a family of 3 estimates
----------------------------------------------
EM Trends pairwise contrasts with transcript abundance against Time
("Time"):
$emtrends
 Genotype Time.trend      SE df lower.CL upper.CL
 EV         -0.00847 0.00341 47 -0.01532 -0.00162
 irTOC1     -0.00259 0.00342 47 -0.00947  0.00428
 irZTL      -0.00461 0.00341 47 -0.01147  0.00224

Confidence level used: 0.95

$contrasts
 contrast       estimate      SE df t.ratio p.value
 EV - irTOC1    -0.00587 0.00483 47 -1.217  0.4491
 EV - irZTL     -0.00385 0.00482 47 -0.800  0.7048
 irTOC1 - irZTL  0.00202 0.00483 47  0.418  0.9082

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

**OPR3:**

```
ANOVA results for transcript abundance ("expr") as response variable:
Response: expr
               Df  Sum Sq Mean Sq  F value    Pr(>F)
Time1           5 28.2907  5.6581 124.6630 < 2.2e-16 ***
Genotype        2  0.5407  0.2703   5.9564 0.0058251 **
Time1:Genotype 10  2.2809  0.2281   5.0254 0.0001454 ***
Residuals      36  1.6340  0.0454
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
EMMeans pairwise contrasts by time point ("Time1"):
contrast       Time1 estimate    SE df t.ratio p.value
 EV - irTOC1    0      0.06775 0.174 36  0.389  0.9200
 EV - irZTL     0      0.06303 0.174 36  0.362  0.9303
 irTOC1 - irZTL 0     -0.00472 0.174 36 -0.027  0.9996
 EV - irTOC1    30     0.39011 0.174 36  2.243  0.0775
 EV - irZTL     30    -0.86445 0.174 36 -4.970  <.0001 ***
```

```
 irTOC1 - irZTL 30    -1.25457 0.174 36 -7.212  <.0001 ***
 EV - irTOC1    60    -0.02744 0.174 36 -0.158  0.9864
 EV - irZTL     60    -0.05000 0.174 36 -0.287  0.9555
 irTOC1 - irZTL 60    -0.02256 0.174 36 -0.130  0.9908
 EV - irTOC1    90     0.07395 0.174 36  0.425  0.9054
 EV - irZTL     90    -0.19938 0.174 36 -1.146  0.4925
 irTOC1 - irZTL 90    -0.27333 0.174 36 -1.571  0.2709
 EV - irTOC1    120    0.32732 0.174 36  1.882  0.1586
 EV - irZTL     120    0.25149 0.174 36  1.446  0.3288
 irTOC1 - irZTL 120   -0.07583 0.174 36 -0.436  0.9008
 EV - irTOC1    180   -0.05917 0.174 36 -0.340  0.9383
 EV - irZTL     180    0.10184 0.174 36  0.585  0.8287
 irTOC1 - irZTL 180    0.16100 0.174 36  0.926  0.6279

P value adjustment: tukey method for comparing a family of 3 estimates
----------------------------------------------
EM Trends pairwise contrasts with transcript abundance against Time
("Time"):
$emtrends
 Genotype Time.trend      SE df lower.CL upper.CL
 EV          0.00417 0.00312 48 -0.00211   0.0104
 irTOC1      0.00495 0.00312 48 -0.00132   0.0112
 irZTL       0.00143 0.00312 48 -0.00484   0.0077

Confidence level used: 0.95

$contrasts
 contrast        estimate      SE df t.ratio p.value
 EV - irTOC1    -0.000784 0.00441 48 -0.178  0.9828
 EV - irZTL      0.002735 0.00441 48  0.620  0.8101
 irTOC1 - irZTL  0.003519 0.00441 48  0.797  0.7064

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

TD:
ANOVA results for transcript abundance ("expr") as response variable:
Response: expr
               Df Sum Sq Mean Sq F value    Pr(>F)
Time1           5 57.587 11.5173 13.1773 9.732e-07 ***
Genotype        2  0.703  0.3513  0.4019   0.67270
Time1:Genotype 10 21.571  2.1571  2.4680   0.02815 *
Residuals      29 25.347  0.8740
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
EMMeans pairwise contrasts by time point ("Time1"):
contrast       Time1 estimate    SE df t.ratio p.value
 EV - irTOC1    0       0.2997 0.763 29  0.393  0.9188
```

```
 EV - irZTL     0       0.2087 1.080 29  0.193  0.9796
 irTOC1 - irZTL 0      -0.0910 1.080 29 -0.084  0.9961
 EV - irTOC1    30      0.1278 0.763 29  0.167  0.9847
 EV - irZTL     30      0.0236 0.763 29  0.031  0.9995
 irTOC1 - irZTL 30     -0.1041 0.763 29 -0.136  0.9898
 EV - irTOC1    60      0.1333 1.080 29  0.123  0.9916
 EV - irZTL     60      0.1803 1.145 29  0.157  0.9864
 irTOC1 - irZTL 60      0.0470 0.853 29  0.055  0.9983
 EV - irTOC1    90      0.6131 0.853 29  0.718  0.7547
 EV - irZTL     90     -1.1260 0.853 29 -1.319  0.3961
 irTOC1 - irZTL 90     -1.7392 0.763 29 -2.278  0.0750 .
 EV - irTOC1    120    -0.6416 0.763 29 -0.841  0.6812
 EV - irZTL     120    -0.3194 0.763 29 -0.418  0.9083
 irTOC1 - irZTL 120     0.3223 0.763 29  0.422  0.9067
 EV - irTOC1    180    -1.8818 0.763 29 -2.465  0.0505 .
 EV - irZTL     180     1.8306 0.853 29  2.145  0.0983 .
 irTOC1 - irZTL 180     3.7124 0.853 29  4.350  0.0004 ***

P value adjustment: tukey method for comparing a family of 3 estimates
----------------------------------------------
EM Trends pairwise contrasts with transcript abundance against Time
("Time"):
$emtrends
 Genotype Time.trend      SE df  lower.CL upper.CL
 EV          0.01504 0.00408 41  0.006805   0.0233
 irTOC1      0.02672 0.00405 41  0.018544   0.0349
 irZTL       0.00948 0.00512 41 -0.000862   0.0198

Confidence level used: 0.95

$contrasts
 contrast       estimate      SE df t.ratio p.value
 EV - irTOC1    -0.01168 0.00575 41 -2.032  0.1173
 EV - irZTL      0.00556 0.00655 41  0.850  0.6747
 irTOC1 - irZTL  0.01724 0.00653 41  2.641  0.0306 *

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

Phytohormones:

**JA:**

```
ANOVA results for ng/g fresh weight (FW) ("Con") as response variable:
Response: Con
               Df   Sum Sq Mean Sq F value    Pr(>F)
Time1           5 24367477 4873495  52.432 < 2.2e-16 ***
Genotype        2  1609492  804746   8.658 0.0003639 ***
Time1:Genotype 10  3185340  318534   3.427 0.0007707 ***
Residuals      90  8365353   92948
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
EMMeans pairwise contrasts by time point ("Time1"):
contrast    Time1 estimate  SE df t.ratio p.value
 EV - irZTL  0        27.34 176 90  0.155  0.9868
 EV - TOC    0        18.43 176 90  0.105  0.9940
 irZTL - TOC 0        -8.92 176 90 -0.051  0.9986
 EV - irZTL  30      -19.79 176 90 -0.112  0.9931
 EV - TOC    30      219.27 176 90  1.246  0.4296
 irZTL - TOC 30      239.06 176 90  1.358  0.3672
 EV - irZTL  60      922.94 176 90  5.243  <.0001 ***
 EV - TOC    60      344.85 176 90  1.959  0.1284
 irZTL - TOC 60     -578.09 176 90 -3.284  0.0041 **
 EV - irZTL  90      758.68 176 90  4.310  0.0001 ***
 EV - TOC    90      214.49 176 90  1.219  0.4454
 irZTL - TOC 90     -544.19 176 90 -3.092  0.0074 **
 EV - irZTL  120     141.95 176 90  0.806  0.7000
 EV - TOC    120     -33.78 176 90 -0.192  0.9799
 irZTL - TOC 120    -175.73 176 90 -0.998  0.5798
 EV - irZTL  180     -56.17 176 90 -0.319  0.9455
 EV - TOC    180    -102.47 176 90 -0.582  0.8300
 irZTL - TOC 180     -46.31 176 90 -0.263  0.9626

P value adjustment: tukey method for comparing a family of 3 estimates
---------------------------------------------
EM Trends pairwise contrasts with JA concentration against Time
("Time"):
$emtrends
 Genotype Time.trend   SE  df lower.CL upper.CL
 EV          -0.6577 1.67 102    -3.97     2.65
 irZTL       -0.0858 1.67 102    -3.40     3.22
 TOC          0.7132 1.67 102    -2.60     4.02

Confidence level used: 0.95

$contrasts
 contrast    estimate   SE  df t.ratio p.value
 EV - irZTL    -0.572 2.36 102 -0.242  0.9682
 EV - TOC      -1.371 2.36 102 -0.581  0.8307
 irZTL - TOC   -0.799 2.36 102 -0.339  0.9388

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

**JA-Ile:**

```
ANOVA results for concentration, ng/g FW ("Con") as response variable:
Response: Con
               Df Sum Sq Mean Sq F value    Pr(>F)
```

```
Time1           5 344078   68816  76.029 < 2.2e-16 ***
Genotype        2  40692   20346  22.479 1.208e-08 ***
Time1:Genotype 10 136584   13658  15.090 1.970e-15 ***
Residuals      90  81461     905
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

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

```
contrast    Time1 estimate   SE df t.ratio p.value
 EV - irZTL  0        1.287 17.4 90  0.074  0.9970
 EV - TOC    0        1.866 17.4 90  0.107  0.9937
 irZTL - TOC 0        0.579 17.4 90  0.033  0.9994
 EV - irZTL  30      26.551 17.4 90  1.529  0.2825
 EV - TOC    30      63.436 17.4 90  3.652  0.0013 **
 irZTL - TOC 30      36.885 17.4 90  2.124  0.0908 .
 EV - irZTL  60      53.726 17.4 90  3.093  0.0074 **
 EV - TOC    60     -81.621 17.4 90 -4.699  <.0001 ***
 irZTL - TOC 60    -135.346 17.4 90 -7.792  <.0001 ***
 EV - irZTL  90     181.194 17.4 90 10.432  <.0001 ***
 EV - TOC    90     139.473 17.4 90  8.030  <.0001 ***
 irZTL - TOC 90     -41.722 17.4 90 -2.402  0.0478 *
 EV - irZTL  120     20.642 17.4 90  1.188  0.4631
 EV - TOC    120      9.062 17.4 90  0.522  0.8609
 irZTL - TOC 120    -11.580 17.4 90 -0.667  0.7834
 EV - irZTL  180      1.589 17.4 90  0.091  0.9954
 EV - TOC    180     -0.869 17.4 90 -0.050  0.9986
 irZTL - TOC 180     -2.458 17.4 90 -0.142  0.9890

P value adjustment: tukey method for comparing a family of 3 estimates
---------------------------------------------
EM Trends pairwise contrasts with JA-Ile concentration against Time
("Time"):
$emtrends
 Genotype Time.trend    SE  df lower.CL upper.CL
 EV          -0.0164 0.209 102   -0.431    0.398
 irZTL       -0.0303 0.209 102   -0.445    0.384
 TOC         -0.0155 0.209 102   -0.430    0.399

Confidence level used: 0.95

$contrasts
 contrast     estimate    SE  df t.ratio p.value
 EV - irZTL   0.013881 0.296 102  0.047  0.9988
 EV - TOC    -0.000876 0.296 102 -0.003  1.0000
 irZTL - TOC -0.014757 0.296 102 -0.050  0.9986

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

## Figure 2:

Outlier removal:

No outliers were removed from this dataset.

Secondary metabolites:

**Nicotine:**

ANOVA results for nicotine concentration Peak area/g FW (“Nicotine”) as response variable:

```
Response: Nicotine
                         Df Sum Sq Mean Sq F value    Pr(>F)
as.factor(Time)           1 368.53  368.53 20.0430 0.0001015 ***
Genotype                  2 577.52  288.76 15.7047 2.154e-05 ***
as.factor(Time):Genotype  2 273.66  136.83  7.4416 0.0023744 **
Residuals                30 551.61   18.39
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by time point in hours (“Time”):

```
contrast       Time estimate   SE df t.ratio p.value
 EV - irTOC1    0      -0.419 2.48 30 -0.169  0.9843
 EV - irZTL     0       2.786 2.48 30  1.125  0.5062
 irTOC1 - irZTL 0       3.205 2.48 30  1.295  0.4091
 EV - irTOC1    72     -9.458 2.48 30 -3.821  0.0018 **
 EV - irZTL     72      6.958 2.48 30  2.811  0.0228
 irTOC1 - irZTL 72     16.417 2.48 30  6.631  <.0001 ***

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

**Caffeoylputrescine:**

ANOVA results for caffeoylputrescine concentration Peak area/g FW (“CP”) as response variable:

```
Response: CP
                         Df Sum Sq Mean Sq F value    Pr(>F)
as.factor(Time)           1 66.115  66.115 48.4932 9.786e-08 ***
Genotype                  2 13.437   6.719  4.9279   0.01411 *
as.factor(Time):Genotype  2  7.559   3.780  2.7723   0.07856 .
Residuals                30 40.902   1.363
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by time point in hours (“Time”):

```
contrast       Time estimate    SE df t.ratio p.value
 EV - irTOC1    0       0.372 0.674 30  0.551  0.8466
 EV - irZTL     0       0.352 0.674 30  0.522  0.8614
 irTOC1 - irZTL 0      -0.020 0.674 30 -0.030  0.9995
 EV - irTOC1    72      2.612 0.674 30  3.874  0.0015 **
 EV - irZTL     72      1.346 0.674 30  1.997  0.1304
 irTOC1 - irZTL 72     -1.266 0.674 30 -1.877  0.1627

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

**Dicaffeoylspermidine:**

ANOVA results for dicaffeoylspermidine concentration Peak area/g FW ("DCS") as response variable:

```
Response: DCS
                         Df  Sum Sq Mean Sq F value    Pr(>F)
as.factor(Time)           1  0.1148  0.1148  0.2928 0.5924389
Genotype                  2  7.4137  3.7069  9.4543 0.0006547 ***
as.factor(Time):Genotype  2  0.3819  0.1909  0.4870 0.6192691
Residuals                30 11.7625  0.3921
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by time point in hours ("Time"):

```
contrast       Time estimate    SE df t.ratio p.value
 EV - irTOC1    0       1.296 0.362 30  3.585  0.0033 **
 EV - irZTL     0       0.988 0.362 30  2.732  0.0274 *
 irTOC1 - irZTL 0      -0.309 0.362 30 -0.853  0.6733
 EV - irTOC1    72      0.865 0.362 30  2.393  0.0585 .
 EV - irZTL     72      0.545 0.362 30  1.507  0.3020
 irTOC1 - irZTL 72     -0.320 0.362 30 -0.886  0.6533

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

Trait correlations:

**Nicotine vs. caffeoylputrescine:**

ANOVA results for nicotine concentration ("Nicotine") as response variable:

```
Response: Nicotine
            Df Sum Sq Mean Sq F value    Pr(>F)
CP           1 137.65  137.65  8.1299  0.007805 **
Genotype     2 694.77  347.39 20.5172 2.425e-06 ***
CP:Genotype  2 430.96  215.48 12.7265 9.952e-05 ***
Residuals   30 507.94   16.93
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by CP level ("CP"):

```
contrast         CP estimate   SE df t.ratio p.value
 EV - irTOC1    1.55   -11.79 2.00 30 -5.896  <.0001 ***
 EV - irZTL     1.55     3.89 1.72 30  2.258  0.0778 .
 irTOC1 - irZTL 1.55    15.68 1.96 30  7.994  <.0001 ***

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

*EM Trends* pairwise contrasts with nicotine against CP concentration ("CP.trend"):

```
$emtrends
```

```
 Genotype CP.trend    SE df lower.CL upper.CL
 EV          1.211 0.498 30    0.195     2.23
 irTOC1      8.297 1.422 30    5.393    11.20
 irZTL       0.492 0.669 30   -0.875     1.86

Confidence level used: 0.95

$contrasts
 contrast       estimate    SE df t.ratio p.value
 EV - irTOC1      -7.086 1.506 30 -4.704  0.0002 ***
 EV - irZTL        0.718 0.834 30  0.861  0.6683
 irTOC1 - irZTL    7.805 1.572 30  4.966  0.0001 ***

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

**Nicotine vs. dicaffeoylspermidine**:

```
ANOVA results nicotine concentration ("Nicotine") as response
variable:
Response: Nicotine
             Df  Sum Sq Mean Sq F value   Pr(>F)
DCS           1   13.31  13.305  0.3730 0.545996
Genotype      2  564.76 282.379  7.9152 0.001736 **
DCS:Genotype  2  122.99  61.494  1.7237 0.195607
Residuals    30 1070.27  35.676
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
EMMeans pairwise contrasts by DCS level ("DCS"):
contrast        DCS estimate   SE df t.ratio p.value
 EV - irTOC1    0.87    -9.85 3.98 30 -2.478  0.0486 *
 EV - irZTL     0.87     5.42 2.81 30  1.931  0.1474
 irTOC1 - irZTL 0.87    15.27 3.88 30  3.941  0.0013 **

P value adjustment: tukey method for comparing a family of 3 estimates
----------------------------------------------
EM Trends pairwise contrasts with nicotine against DCS concentration
("DCS.trend"):
$emtrends
 Genotype DCS.trend   SE df lower.CL upper.CL
 EV          -0.631 1.90 30     -4.5     3.24
 irTOC1      11.405 6.27 30     -1.4    24.21
 irZTL       -1.058 5.00 30    -11.3     9.15

Confidence level used: 0.95

$contrasts
 contrast       estimate   SE df t.ratio p.value
 EV - irTOC1     -12.036 6.55 30 -1.837  0.1750
```

```
 EV - irZTL        0.427 5.35 30  0.080  0.9965
 irTOC1 - irZTL   12.463 8.02 30  1.554  0.2809

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

# Figures 3, 4, and S1:

Outlier removal:

One data point (21h control treatment, irTOC1 replicate 3) did not produce clear peaks for nicotine and CP and was removed before analysis.

Secondary metabolite concentrations (Figure 3b,c,d):

**Nicotine:**

ANOVA results for nicotine concentration in ug/mL ("Nic_ug_mL") as response variable:

```
Response: Nic_ug_mL
                              Df Sum Sq Mean Sq  F value    Pr(>F)
Genotype                       1 100135  100135 189.9925 < 2.2e-16 ***
Treatment                      1 339606  339606 644.3537 < 2.2e-16 ***
Timepoint                      7  75975   10854  20.5930 < 2.2e-16 ***
Genotype:Treatment             1  10308   10308  19.5574 2.272e-05 ***
Genotype:Timepoint             7  69089    9870  18.7266 2.408e-16 ***
Treatment:Timepoint            5  17581    3516   6.6713 1.786e-05 ***
Genotype:Treatment:Timepoint   5  13854    2771   5.2571 0.0002248 ***
Residuals                    112  59029     527
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in hours ("Treatment","Timepoint"):

```
contrast    Treatment Timepoint estimate   SE  df t.ratio p.value
 EV - irTOC1 Control   0            -33.1 14.5 112  -2.277 0.0247 *
 EV - irTOC1 Control   5            -29.8 14.5 112  -2.052 0.0425 *
 EV - irTOC1 W+OS      5             -5.6 14.5 112  -0.386 0.7002
 EV - irTOC1 Control   9            -39.0 14.5 112  -2.686 0.0083 **
 EV - irTOC1 W+OS      9            -16.6 14.5 112  -1.141 0.2565
 EV - irTOC1 Control   21           -60.2 14.5 112  -4.149 0.0001 ***
 EV - irTOC1 W+OS      21           -34.3 14.5 112  -2.361 0.0199 *
 EV - irTOC1 Control   33           -32.2 14.5 112  -2.216 0.0287 *
 EV - irTOC1 W+OS      33           -26.4 14.5 112  -1.820 0.0714 .
 EV - irTOC1 Control   45           -22.8 14.5 112  -1.571 0.1190
 EV - irTOC1 W+OS      45           -86.5 14.5 112  -5.961 <.0001 ***
 EV - irTOC1 Control   69           -37.2 14.5 112  -2.561 0.0118 *
 EV - irTOC1 W+OS      69          -113.8 14.5 112  -7.836 <.0001 ***
 EV - irTOC1 W+OS      93          -211.4 14.5 112 -14.556 <.0001 ***
```

**Caffeoylputrescine:**

ANOVA results for caffeoylputrescine concentration in ug/mL ("CP_ug_mL") as response variable:

```
Response: CP_ug_mL
                              Df Sum Sq Mean Sq   F value    Pr(>F)
```

```
Genotype                      1  348.5   348.5  415.1142 < 2.2e-16
***
Treatment                     1 3549.8  3549.8 4227.7746 < 2.2e-16
***
Timepoint                     7 2107.2   301.0  358.5205 < 2.2e-16
***
Genotype:Treatment            1  114.2   114.2  136.0550 < 2.2e-16
***
Genotype:Timepoint            7   50.9     7.3    8.6647 1.804e-08
***
Treatment:Timepoint           5  664.2   132.8  158.2216 < 2.2e-16
***
Genotype:Treatment:Timepoint  5   27.0     5.4    6.4304 2.735e-05
***
Residuals                   112   94.0     0.8
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in hours ("Treatment","Timepoint"):

```
contrast    Treatment Timepoint estimate   SE  df t.ratio p.value
 EV - irTOC1 Control   0             1.10 0.58 112  1.905  0.0593 .
 EV - irTOC1 Control   5             1.15 0.58 112  1.981  0.0500 *
 EV - irTOC1 W+OS      5             3.10 0.58 112  5.347  <.0001 ***
 EV - irTOC1 Control   9             1.35 0.58 112  2.327  0.0217 *
 EV - irTOC1 W+OS      9             3.75 0.58 112  6.479  <.0001 ***
 EV - irTOC1 Control   21            1.04 0.58 112  1.795  0.0754 .
 EV - irTOC1 W+OS      21            2.95 0.58 112  5.097  <.0001 ***
 EV - irTOC1 Control   33            1.11 0.58 112  1.917  0.0578 .
 EV - irTOC1 W+OS      33            3.46 0.58 112  5.965  <.0001 ***
 EV - irTOC1 Control   45            0.62 0.58 112  1.070  0.2867
 EV - irTOC1 W+OS      45            7.94 0.58 112 13.708  <.0001 ***
 EV - irTOC1 Control   69            3.07 0.58 112  5.299  <.0001 ***
 EV - irTOC1 W+OS      69            6.13 0.58 112 10.577  <.0001 ***
 EV - irTOC1 W+OS      93            7.40 0.58 112 12.766  <.0001 ***
```

**Dicaffeoylspermidine:**

ANOVA results for dicaffeoylspermidine concentration in ug/mL ("DCS_ug_mL") as response variable:

```
Response: DCS_ug_mL
                              Df Sum Sq Mean Sq  F value    Pr(>F)
Genotype                       1  13602 13602.0 633.0032 < 2.2e-16 ***
Treatment                      1  20440 20440.3 951.2406 < 2.2e-16 ***
Timepoint                      7  42553  6079.0 282.9017 < 2.2e-16 ***
Genotype:Treatment             1    711   710.9  33.0854 7.773e-08 ***
Genotype:Timepoint             7    701   100.2   4.6616 0.0001298 ***
Treatment:Timepoint            5   9842  1968.4  91.6035 < 2.2e-16 ***
Genotype:Treatment:Timepoint   5   1092   218.4  10.1659 4.771e-08 ***
```

```
Residuals                    112   2407    21.5
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
EMMeans pairwise contrasts by treatment and time point in hours
("Treatment","Timepoint"):
contrast    Treatment Timepoint estimate   SE  df t.ratio p.value
 EV - irTOC1 Control   0             11.8 2.93 112  4.025  0.0001 ***
 EV - irTOC1 Control   5             11.5 2.93 112  3.916  0.0002 ***
 EV - irTOC1 W+OS      5             26.4 2.93 112  8.996  <.0001 ***
 EV - irTOC1 Control   9             27.4 2.93 112  9.343  <.0001 ***
 EV - irTOC1 W+OS      9             15.3 2.93 112  5.206  <.0001 ***
 EV - irTOC1 Control   21            15.6 2.93 112  5.330  <.0001 ***
 EV - irTOC1 W+OS      21            27.1 2.93 112  9.252  <.0001 ***
 EV - irTOC1 Control   33            13.6 2.93 112  4.636  <.0001 ***
 EV - irTOC1 W+OS      33            14.1 2.93 112  4.821  <.0001 ***
 EV - irTOC1 Control   45            10.3 2.93 112  3.518  0.0006 ***
 EV - irTOC1 W+OS      45            29.4 2.93 112 10.029  <.0001 ***
 EV - irTOC1 Control   69            16.2 2.93 112  5.541  <.0001 ***
 EV - irTOC1 W+OS      69            39.9 2.93 112 13.614  <.0001 ***
 EV - irTOC1 W+OS      93            17.3 2.93 112  5.913  <.0001 ***
```

Secondary metabolite $^{15}$N incorporation rates (Figure **3e,f,g**):

**Nicotine:**

```
ANOVA results for nicotine 15N %At incorporation rate
("Nic_15N_incorp") as response variable:
Response: Nic_15N_incorp
                              Df Sum Sq Mean Sq F value    Pr(>F)
Genotype                       1  61.05  61.048 50.9327 1.044e-10 ***
Treatment                      1  23.06  23.064 19.2423 2.632e-05 ***
Timepoint                      7 366.31  52.330 43.6596 < 2.2e-16 ***
Genotype:Treatment             1   3.85   3.851  3.2128  0.075786 .
Genotype:Timepoint             7  24.73   3.533  2.9473  0.007188 **
Treatment:Timepoint            5 222.30  44.461 37.0939 < 2.2e-16 ***
Genotype:Treatment:Timepoint   5   5.99   1.198  0.9996  0.421510
Residuals                    111 133.04   1.199
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
EMMeans pairwise contrasts by treatment and time point in hours
("Treatment","Timepoint"):
contrast    Treatment Timepoint estimate    SE  df t.ratio p.value
 EV - irTOC1 Control   0            -1.48 0.692 111 -2.137  0.0348 *
 EV - irTOC1 Control   5            -2.36 0.692 111 -3.408  0.0009 ***
 EV - irTOC1 W+OS      5            -1.66 0.692 111 -2.397  0.0182 *
 EV - irTOC1 Control   9            -2.54 0.692 111 -3.668  0.0004 ***
 EV - irTOC1 W+OS      9            -2.92 0.692 111 -4.217  0.0001 ***
```

```
 EV - irTOC1 Control   21           -0.84 0.734 111 -1.144  0.2552
 EV - irTOC1 W+OS      21           -1.70 0.692 111 -2.455  0.0156 *
 EV - irTOC1 Control   33           -0.88 0.692 111 -1.271  0.2064
 EV - irTOC1 W+OS      33           -1.84 0.692 111 -2.657  0.0090 **
 EV - irTOC1 Control   45            0.66 0.692 111  0.953  0.3426
 EV - irTOC1 W+OS      45           -1.08 0.692 111 -1.560  0.1217
 EV - irTOC1 Control   69            0.56 0.692 111  0.809  0.4204
 EV - irTOC1 W+OS      69           -1.46 0.692 111 -2.109  0.0372 *
 EV - irTOC1 W+OS      93           -0.88 0.692 111 -1.271  0.2064
```

**Caffeoylputrescine:**

ANOVA results for caffeoylputrescine $^{15}$N %At incorporation rate ("CP_15N_incorp") as response variable:

```
Response: CP_15N_ incorp
                             Df  Sum Sq Mean Sq  F value    Pr(>F)
Genotype                      1 1432.62 1432.62 931.3101 < 2.2e-16
***
Treatment                     1 1330.78 1330.78 865.1058 < 2.2e-16
***
Timepoint                     7 1954.63  279.23 181.5215 < 2.2e-16
***
Genotype:Treatment            1   62.49   62.49  40.6255 4.334e-09
***
Genotype:Timepoint            7  218.71   31.24  20.3107 < 2.2e-16
***
Treatment:Timepoint           5   42.37    8.47   5.5092 0.0001437
***
Genotype:Treatment:Timepoint  5  427.28   85.46  55.5526 < 2.2e-16
***
Residuals                   111  170.75    1.54
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in hours ("Treatment","Timepoint"):

```
contrast    Treatment Timepoint estimate    SE  df t.ratio p.value
 EV - irTOC1 Control   0             0.00 0.784 111  0.000  1.0000
 EV - irTOC1 Control   5             0.00 0.784 111  0.000  1.0000
 EV - irTOC1 W+OS      5            10.68 0.784 111 13.615  <.0001 ***
 EV - irTOC1 Control   9            12.46 0.784 111 15.884  <.0001 ***
 EV - irTOC1 W+OS      9             2.76 0.784 111  3.519  0.0006 ***
 EV - irTOC1 Control   21           13.75 0.832 111 16.526  <.0001 ***
 EV - irTOC1 W+OS      21            4.38 0.784 111  5.584  <.0001 ***
 EV - irTOC1 Control   33           13.30 0.784 111 16.955  <.0001 ***
 EV - irTOC1 W+OS      33            1.74 0.784 111  2.218  0.0286 *
 EV - irTOC1 Control   45            8.80 0.784 111 11.218  <.0001 ***
 EV - irTOC1 W+OS      45            4.14 0.784 111  5.278  <.0001 ***
 EV - irTOC1 Control   69            6.92 0.784 111  8.822  <.0001 ***
```

```
 EV - irTOC1 W+OS      69            5.98 0.784 111  7.623  <.0001 ***
 EV - irTOC1 W+OS      93            6.16 0.784 111  7.853  <.0001 ***
```

**Dicaffeoylspermidine:**

ANOVA results for dicaffeoylspermidine $^{15}$N %At incorporation rate ("DCS_15N_incorp") as response variable:

```
Response: DCS_15N_incorp
                              Df Sum Sq Mean Sq  F value    Pr(>F)
Genotype                       1 442.15  442.15 258.5097 < 2.2e-16 ***
Treatment                      1  65.83   65.83  38.4874 9.510e-09 ***
Timepoint                      7 783.95  111.99  65.4780 < 2.2e-16 ***
Genotype:Treatment             1   0.06    0.06   0.0376   0.84663
Genotype:Timepoint             7  41.80    5.97   3.4912   0.00201 **
Treatment:Timepoint            5 160.61   32.12  18.7808 1.607e-13 ***
Genotype:Treatment:Timepoint   5  22.74    4.55   2.6595   0.02603 *
Residuals                    112 191.56    1.71
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in hours ("Treatment","Timepoint"):

```
contrast    Treatment Timepoint estimate    SE  df t.ratio p.value
 EV - irTOC1 Control   0            1.74 0.827 112 2.104   0.0376 *
 EV - irTOC1 Control   5            2.28 0.827 112 2.756   0.0068 **
 EV - irTOC1 W+OS      5            3.32 0.827 112 4.014   0.0001 ***
 EV - irTOC1 Control   9            4.94 0.827 112 5.972   <.0001 ***
 EV - irTOC1 W+OS      9            2.26 0.827 112 2.732   0.0073 **
 EV - irTOC1 Control   21           5.38 0.827 112 6.504   <.0001 ***
 EV - irTOC1 W+OS      21           5.46 0.827 112 6.601   <.0001 ***
 EV - irTOC1 Control   33           3.70 0.827 112 4.473   <.0001 ***
 EV - irTOC1 W+OS      33           3.02 0.827 112 3.651   0.0004 ***
 EV - irTOC1 Control   45           3.40 0.827 112 4.111   0.0001 ***
 EV - irTOC1 W+OS      45           6.08 0.827 112 7.351   <.0001 ***
 EV - irTOC1 Control   69           3.74 0.827 112 4.522   <.0001 ***
 EV - irTOC1 W+OS      69           2.16 0.827 112 2.611   0.0103 *
 EV - irTOC1 W+OS      93           2.28 0.827 112 2.756   0.0068 **
```

$^{15}$N-labeled secondary metabolite concentrations (Figure 3h,j,k):

**Nicotine:**

ANOVA results for labeled nicotine concentration in ug/mL ("Nic_15N") as response variable:

```
Response: Nic_15N
                              Df Sum Sq Mean Sq  F value    Pr(>F)
Genotype                       1 4053.3  4053.3 193.2173 < 2.2e-16 ***
Treatment                      1 7179.7  7179.7 342.2481 < 2.2e-16 ***
Timepoint                      7 4277.5   611.1  29.1287 < 2.2e-16 ***
```

```
Genotype:Treatment             1  523.6   523.6  24.9606 2.196e-06 ***
Genotype:Timepoint             7  722.7   103.2   4.9214 7.184e-05 ***
Treatment:Timepoint            5 2266.9   453.4  21.6125 4.706e-15 ***
Genotype:Treatment:Timepoint   5  372.5    74.5   3.5517  0.005122 **
Residuals                    111 2328.6    21.0
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in hours ("Treatment","Timepoint"):

```
contrast    Treatment Timepoint estimate   SE  df t.ratio p.value
 EV - irTOC1 Control   0            -5.73 2.90 111  -1.978 0.0504 .
 EV - irTOC1 Control   5            -9.65 2.90 111  -3.331 0.0012 **
 EV - irTOC1 W+OS      5            -6.29 2.90 111  -2.173 0.0319 *
 EV - irTOC1 Control   9           -12.46 2.90 111  -4.300 <.0001 ***
 EV - irTOC1 W+OS      9           -11.62 2.90 111  -4.012 0.0001 ***
 EV - irTOC1 Control   21           -9.15 3.07 111  -2.980 0.0035 **
 EV - irTOC1 W+OS      21           -9.75 2.90 111  -3.366 0.0010 ***
 EV - irTOC1 Control   33           -5.74 2.90 111  -1.981 0.0501 .
 EV - irTOC1 W+OS      33          -10.67 2.90 111  -3.684 0.0004 ***
 EV - irTOC1 Control   45           -1.17 2.90 111  -0.404 0.6869
 EV - irTOC1 W+OS      45          -15.51 2.90 111  -5.353 <.0001 ***
 EV - irTOC1 Control   69           -4.01 2.90 111  -1.386 0.1687
 EV - irTOC1 W+OS      69          -18.44 2.90 111  -6.365 <.0001 ***
 EV - irTOC1 W+OS      93          -29.40 2.90 111 -10.148 <.0001 ***
```

**Caffeoylputrescine:**

ANOVA results for labeled caffeoylputrescine concentration in ug/mL ("CP_15N") as response variable:

```
Response: CP_15N
                              Df Sum Sq Mean Sq  F value    Pr(>F)
Genotype                       1 19.874  19.874  900.120 < 2.2e-16 ***
Treatment                      1 77.741  77.741 3520.944 < 2.2e-16 ***
Timepoint                      7 63.113   9.016  408.349 < 2.2e-16 ***
Genotype:Treatment             1  9.144   9.144  414.156 < 2.2e-16 ***
Genotype:Timepoint             7  7.645   1.092   49.465 < 2.2e-16 ***
Treatment:Timepoint            5 18.637   3.727  168.818 < 2.2e-16 ***
Genotype:Treatment:Timepoint   5  1.952   0.390   17.680 7.458e-13 ***
Residuals                    111  2.451   0.022
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in hours ("Treatment","Timepoint"):

```
contrast    Treatment Timepoint estimate     SE  df t.ratio p.value
 EV - irTOC1 Control   0            0.000 0.0940 111  0.000  1.0000
 EV - irTOC1 Control   5            0.000 0.0940 111  0.000  1.0000
```

```
 EV - irTOC1 W+OS      5            0.664 0.0940 111  7.068  <.0001
***
 EV - irTOC1 Control   9            0.271 0.0940 111  2.887  0.0047 **
 EV - irTOC1 W+OS      9            0.561 0.0940 111  5.968  <.0001
***
 EV - irTOC1 Control   21           0.353 0.0997 111  3.537  0.0006
***
 EV - irTOC1 W+OS      21           0.931 0.0940 111  9.912  <.0001
***
 EV - irTOC1 Control   33           0.286 0.0940 111  3.044  0.0029 **
 EV - irTOC1 W+OS      33           0.565 0.0940 111  6.012  <.0001
***
 EV - irTOC1 Control   45           0.235 0.0940 111  2.498  0.0139 *
 EV - irTOC1 W+OS      45           1.773 0.0940 111 18.865  <.0001
***
 EV - irTOC1 Control   69           0.627 0.0940 111  6.673  <.0001
***
 EV - irTOC1 W+OS      69           2.075 0.0940 111 22.083  <.0001
***
 EV - irTOC1 W+OS      93           2.355 0.0940 111 25.057  <.0001
***
```

**Dicaffeoylspermidine:**

ANOVA results for labeled dicaffeoylspermidine concentration in ug/mL (“DCS_15N”) as response variable:

```
Response: DCS_15N
                            Df Sum Sq Mean Sq F value    Pr(>F)
Genotype                     1 261.29 261.293 373.061 < 2.2e-16 ***
Treatment                    1 275.94 275.945 393.979 < 2.2e-16 ***
Timepoint                    7 777.78 111.111 158.639 < 2.2e-16 ***
Genotype:Treatment           1  25.27  25.273  36.084 2.397e-08 ***
Genotype:Timepoint           7  49.26   7.037  10.047 1.063e-09 ***
Treatment:Timepoint          5 185.61  37.121  53.000 < 2.2e-16 ***
Genotype:Treatment:Timepoint 5  39.19   7.839  11.191 9.220e-09 ***
Residuals                  112  78.45   0.700
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

---

*EMMeans* pairwise contrasts by treatment and time point in hours (“Treatment”,“Timepoint”):

```
contrast    Treatment Timepoint estimate    SE  df t.ratio p.value
 EV - irTOC1 Control   0            0.560 0.529 112  1.058  0.2923
 EV - irTOC1 Control   5            0.832 0.529 112  1.573  0.1186
 EV - irTOC1 W+OS      5            2.265 0.529 112  4.279  <.0001 ***
 EV - irTOC1 Control   9            2.805 0.529 112  5.299  <.0001 ***
 EV - irTOC1 W+OS      9            1.200 0.529 112  2.267  0.0253 *
 EV - irTOC1 Control   21           2.512 0.529 112  4.746  <.0001 ***
 EV - irTOC1 W+OS      21           3.625 0.529 112  6.848  <.0001 ***
```

```
 EV - irTOC1 Control   33          1.881 0.529 112  3.553  0.0006 ***
 EV - irTOC1 W+OS      33          1.191 0.529 112  2.251  0.0263 *
 EV - irTOC1 Control   45          1.784 0.529 112  3.370  0.0010 ***
 EV - irTOC1 W+OS      45          7.131 0.529 112 13.472  <.0001 ***
 EV - irTOC1 Control   69          2.804 0.529 112  5.298  <.0001 ***
 EV - irTOC1 W+OS      69          5.721 0.529 112 10.809  <.0001 ***
 EV - irTOC1 W+OS      93          3.941 0.529 112  7.446  <.0001 ***
```

Secondary metabolite total pools (Figure S1b,c,d):

**Nicotine:**

```
ANOVA results for whole-leaf nicotine pools ("Nic_total_pools") as
response variable:
Response: Nic_total_pools
                            Df  Sum Sq Mean Sq  F value    Pr(>F)
Genotype                     1  546217  546217  69.2627 2.346e-13
***
Treatment                    1 1479607 1479607 187.6207 < 2.2e-16
***
Timepoint                    7 2863564  409081  51.8732 < 2.2e-16
***
Genotype:Treatment           1   37748   37748   4.7867   0.03076 *
Genotype:Timepoint           7  267491   38213   4.8456 8.463e-05
***
Treatment:Timepoint          5  310473   62095   7.8739 2.197e-06
***
Genotype:Treatment:Timepoint   5  150131   30026   3.8075   0.00319 **
Residuals                    112  883250    7886
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
EMMeans pairwise contrasts by treatment and time point in hours
("Treatment","Timepoint"):
contrast    Treatment Timepoint estimate   SE  df t.ratio p.value
 EV - irTOC1 Control   0            -24.4 56.2 112 -0.435  0.6645
 EV - irTOC1 Control   5            -42.1 56.2 112 -0.750  0.4550
 EV - irTOC1 W+OS      5            -29.4 56.2 112 -0.524  0.6016
 EV - irTOC1 Control   9            -45.5 56.2 112 -0.810  0.4194
 EV - irTOC1 W+OS      9            -64.2 56.2 112 -1.143  0.2555
 EV - irTOC1 Control   21          -258.7 56.2 112 -4.606  <.0001 ***
 EV - irTOC1 W+OS      21           -20.2 56.2 112 -0.359  0.7202
 EV - irTOC1 Control   33           -62.5 56.2 112 -1.113  0.2679
 EV - irTOC1 W+OS      33           -85.0 56.2 112 -1.513  0.1330
 EV - irTOC1 Control   45          -121.0 56.2 112 -2.155  0.0333 *
 EV - irTOC1 W+OS      45          -199.8 56.2 112 -3.557  0.0006 ***
 EV - irTOC1 Control   69           -90.3 56.2 112 -1.607  0.1108
 EV - irTOC1 W+OS      69          -331.1 56.2 112 -5.896  <.0001 ***
 EV - irTOC1 W+OS      93          -374.7 56.2 112 -6.671  <.0001 ***
```

**Caffeoylputrescine:**

ANOVA results for whole-leaf caffeoylputrescine pools
("CP_total_pools") as response variable:

```
Response: CP_total_pools
                              Df  Sum Sq Mean Sq   F value    Pr(>F)
Genotype                       1  1076.1  1076.1  150.2229 < 2.2e-16
***
Treatment                      1 12834.3 12834.3 1791.6866 < 2.2e-16
***
Timepoint                      7 12202.0  1743.1  243.3444 < 2.2e-16
***
Genotype:Treatment             1   393.1   393.1   54.8782 2.584e-11
***
Genotype:Timepoint             7   422.9    60.4    8.4347 2.925e-08
***
Treatment:Timepoint            5  3564.2   712.8   99.5131 < 2.2e-16
***
Genotype:Treatment:Timepoint   5   141.4    28.3    3.9470  0.002468
**
Residuals                    112   802.3     7.2
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in hours
("Treatment","Timepoint"):

```
contrast    Treatment Timepoint estimate   SE  df t.ratio p.value
 EV - irTOC1 Control   0            1.281 1.69 112  0.757  0.4509
 EV - irTOC1 Control   5            1.822 1.69 112  1.076  0.2841
 EV - irTOC1 W+OS      5            3.921 1.69 112  2.316  0.0224 *
 EV - irTOC1 Control   9            2.201 1.69 112  1.300  0.1962
 EV - irTOC1 W+OS      9            4.215 1.69 112  2.490  0.0142 *
 EV - irTOC1 Control   21           0.182 1.69 112  0.107  0.9146
 EV - irTOC1 W+OS      21           5.797 1.69 112  3.425  0.0009 ***
 EV - irTOC1 Control   33           1.898 1.69 112  1.121  0.2645
 EV - irTOC1 W+OS      33           5.467 1.69 112  3.230  0.0016 **
 EV - irTOC1 Control   45           0.462 1.69 112  0.273  0.7853
 EV - irTOC1 W+OS      45          14.777 1.69 112  8.730  <.0001 ***
 EV - irTOC1 Control   69           7.508 1.69 112  4.436  <.0001 ***
 EV - irTOC1 W+OS      69           9.944 1.69 112  5.875  <.0001 ***
 EV - irTOC1 W+OS      93          18.152 1.69 112 10.723  <.0001 ***
```

**Dicaffeoylspermidine:**

ANOVA results for whole-leaf dicaffeoylspermidine pools
("DCS_total_pools") as response variable:

```
Response: DCS_total_pools
                              Df Sum Sq Mean Sq  F value    Pr(>F)
Genotype                       1  36528   36528 177.3054 < 2.2e-16 ***
```

```
Treatment                      1  87931   87931 426.8139 < 2.2e-16 ***
Timepoint                      7 285038   40720 197.6511 < 2.2e-16 ***
Genotype:Treatment             1   3377    3377  16.3935 9.508e-05 ***
Genotype:Timepoint             7   4497     642   3.1181 0.0048106 **
Treatment:Timepoint            5  48778    9756  47.3528 < 2.2e-16 ***
Genotype:Treatment:Timepoint   5   4973     995   4.8277 0.0004912 ***
Residuals                    112  23074     206
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
EMMeans pairwise contrasts by treatment and time point in hours
("Treatment","Timepoint"):
contrast    Treatment Timepoint estimate   SE  df t.ratio p.value
 EV - irTOC1 Control   0            14.28 9.08 112 1.573   0.1186
 EV - irTOC1 Control   5            18.55 9.08 112 2.043   0.0434 *
 EV - irTOC1 W+OS      5            33.57 9.08 112 3.698   0.0003 ***
 EV - irTOC1 Control   9            44.66 9.08 112 4.919   <.0001 ***
 EV - irTOC1 W+OS      9            16.66 9.08 112 1.836   0.0691 .
 EV - irTOC1 Control   21            6.50 9.08 112 0.717   0.4752
 EV - irTOC1 W+OS      21           43.41 9.08 112 4.782   <.0001 ***
 EV - irTOC1 Control   33           23.78 9.08 112 2.620   0.0100 **
 EV - irTOC1 W+OS      33           21.99 9.08 112 2.423   0.0170 *
 EV - irTOC1 Control   45            8.39 9.08 112 0.925   0.3571
 EV - irTOC1 W+OS      45           56.06 9.08 112 6.175   <.0001 ***
 EV - irTOC1 Control   69           41.22 9.08 112 4.540   <.0001 ***
 EV - irTOC1 W+OS      69           74.36 9.08 112 8.192   <.0001 ***
 EV - irTOC1 W+OS      93           48.84 9.08 112 5.381   <.0001 ***
```

Secondary metabolite total $^{15}$N-labeled pools (Figure **S1e,f,g**):

**Nicotine:**

```
ANOVA results for whole-leaf 15N-labeled nicotine pools
("Nic_15N_pools") as response variable:
Response: Nic_15N_pools
                              Df Sum Sq Mean Sq  F value    Pr(>F)
Genotype                       1  17394 17394.2 125.1303 < 2.2e-16 ***
Treatment                      1  25104 25103.9 180.5923 < 2.2e-16 ***
Timepoint                      7  61616  8802.3  63.3219 < 2.2e-16 ***
Genotype:Treatment             1   1870  1869.9  13.4516 0.0003775 ***
Genotype:Timepoint             7   2721   388.8   2.7966 0.0101831 *
Treatment:Timepoint            5  12837  2567.3  18.4687 2.603e-13 ***
Genotype:Treatment:Timepoint   5   3043   608.6   4.3784 0.0011239 **
Residuals                    111  15430   139.0
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
EMMeans pairwise contrasts by treatment and time point in hours
("Treatment","Timepoint"):
```

```
contrast    Treatment Timepoint estimate   SE  df t.ratio p.value
 EV - irTOC1 Control   0            -5.64 7.46 111 -0.756  0.4511
 EV - irTOC1 Control   5           -14.62 7.46 111 -1.960  0.0525 .
 EV - irTOC1 W+OS      5           -10.83 7.46 111 -1.453  0.1490
 EV - irTOC1 Control   9           -17.40 7.46 111 -2.334  0.0214 *
 EV - irTOC1 W+OS      9           -21.61 7.46 111 -2.898  0.0045 **
 EV - irTOC1 Control   21          -35.44 7.91 111 -4.481  <.0001 ***
 EV - irTOC1 W+OS      21          -10.66 7.46 111 -1.430  0.1555
 EV - irTOC1 Control   33          -10.28 7.46 111 -1.378  0.1709
 EV - irTOC1 W+OS      33          -24.50 7.46 111 -3.286  0.0014 **
 EV - irTOC1 Control   45          -12.58 7.46 111 -1.687  0.0945 .
 EV - irTOC1 W+OS      45          -34.53 7.46 111 -4.631  <.0001 ***
 EV - irTOC1 Control   69           -9.63 7.46 111 -1.291  0.1993
 EV - irTOC1 W+OS      69          -49.93 7.46 111 -6.696  <.0001 ***
 EV - irTOC1 W+OS      93          -53.08 7.46 111 -7.118  <.0001 ***
```

**Caffeoylputrescine:**

ANOVA results for whole-leaf $^{15}$N-labeled caffeoylputrescine pools ("CP_15N_pools") as response variable:

```
Response: CP_15N_pools
                              Df Sum Sq Mean Sq  F value    Pr(>F)
Genotype                       1  69.32  69.315  534.034 < 2.2e-16 ***
Treatment                      1 291.43 291.429 2245.286 < 2.2e-16 ***
Timepoint                      7 333.29  47.614  366.833 < 2.2e-16 ***
Genotype:Treatment             1  32.52  32.516  250.517 < 2.2e-16 ***
Genotype:Timepoint             7  46.58   6.655   51.270 < 2.2e-16 ***
Treatment:Timepoint            5  96.80  19.359  149.153 < 2.2e-16 ***
Genotype:Treatment:Timepoint   5   8.46   1.692   13.037 5.535e-10 ***
Residuals                    111  14.41   0.130
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in hours ("Treatment","Timepoint"):

```
contrast    Treatment Timepoint estimate    SE  df t.ratio p.value
 EV - irTOC1 Control   0            0.000 0.228 111  0.000  1.0000
 EV - irTOC1 Control   5            0.000 0.228 111  0.000  1.0000
 EV - irTOC1 W+OS      5            0.886 0.228 111  3.889  0.0002 ***
 EV - irTOC1 Control   9            0.434 0.228 111  1.906  0.0592 .
 EV - irTOC1 W+OS      9            0.680 0.228 111  2.982  0.0035 **
 EV - irTOC1 Control   21           0.403 0.242 111  1.667  0.0982 .
 EV - irTOC1 W+OS      21           1.520 0.228 111  6.670  <.0001 ***
 EV - irTOC1 Control   33           0.485 0.228 111  2.130  0.0354 *
 EV - irTOC1 W+OS      33           0.910 0.228 111  3.994  0.0001 ***
 EV - irTOC1 Control   45           0.332 0.228 111  1.456  0.1483
 EV - irTOC1 W+OS      45           3.415 0.228 111 14.988  <.0001 ***
 EV - irTOC1 Control   69           1.557 0.228 111  6.834  <.0001 ***
 EV - irTOC1 W+OS      69           4.103 0.228 111 18.005  <.0001 ***
```

```
 EV - irTOC1 W+OS      93           5.263 0.228 111 23.100  <.0001 ***
```

**Dicaffeoylspermidine:**

ANOVA results for whole-leaf $^{15}$N-labeled dicaffeoylspermidine pools ("DCS_15N_pools") as response variable:

```
Response: DCS_15N_pools
                              Df Sum Sq Mean Sq  F value    Pr(>F)
Genotype                       1  838.3  838.25 197.4213 < 2.2e-16 ***
Treatment                      1 1208.9 1208.87 284.7057 < 2.2e-16 ***
Timepoint                      7 4079.9  582.84 137.2683 < 2.2e-16 ***
Genotype:Treatment             1  111.8  111.82  26.3349 1.218e-06 ***
Genotype:Timepoint             7  276.2   39.46   9.2924 4.913e-09 ***
Treatment:Timepoint            5  830.5  166.09  39.1174 < 2.2e-16 ***
Genotype:Treatment:Timepoint   5  163.7   32.74   7.7099 2.914e-06 ***
Residuals                    112  475.6    4.25
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in hours ("Treatment","Timepoint"):

```
contrast    Treatment Timepoint estimate  SE  df t.ratio p.value
 EV - irTOC1 Control   0            0.669 1.3 112  0.513  0.6087
 EV - irTOC1 Control   5            1.294 1.3 112  0.993  0.3229
 EV - irTOC1 W+OS      5            2.874 1.3 112  2.206  0.0295 *
 EV - irTOC1 Control   9            4.439 1.3 112  3.406  0.0009 ***
 EV - irTOC1 W+OS      9            1.527 1.3 112  1.172  0.2439
 EV - irTOC1 Control   21           2.462 1.3 112  1.889  0.0615 .
 EV - irTOC1 W+OS      21           5.497 1.3 112  4.218  0.0001 ***
 EV - irTOC1 Control   33           3.332 1.3 112  2.557  0.0119 *
 EV - irTOC1 W+OS      33           2.014 1.3 112  1.545  0.1251
 EV - irTOC1 Control   45           2.494 1.3 112  1.914  0.0582 .
 EV - irTOC1 W+OS      45          14.108 1.3 112 10.826  <.0001 ***
 EV - irTOC1 Control   69           7.056 1.3 112  5.414  <.0001 ***
 EV - irTOC1 W+OS      69          11.331 1.3 112  8.694  <.0001 ***
 EV - irTOC1 W+OS      93           9.418 1.3 112  7.226  <.0001 ***
```

Normalized differences in concentration vs. Nicotine (Figure 4c,d):

**Nicotine vs. caffeoylputrescine:**

ANOVA results for normalized differences in concentration between nicotine and caffeoylputrescine ("Norm_Nic_CP_conc") as response variable:

```
Response: Norm_Nic_CP_conc
                              Df   Sum Sq  Mean Sq   F value    Pr(>F)
Genotype                       1 0.023170 0.023170  494.4796 < 2.2e-16
***
```

```
Treatment                      1 0.085159 0.085159 1817.4079 < 2.2e-16
***
Timepoint                      7 0.050061 0.007152  152.6242 < 2.2e-16
***
Genotype:Treatment             1 0.006783 0.006783  144.7648 < 2.2e-16
***
Genotype:Timepoint             7 0.007433 0.001062   22.6622 < 2.2e-16
***
Treatment:Timepoint            5 0.013925 0.002785   59.4348 < 2.2e-16
***
Genotype:Treatment:Timepoint   5 0.001593 0.000319    6.7999 1.423e-05
***
Residuals                    112 0.005248 0.000047
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in hours ("Treatment","Timepoint"):

```
contrast    Treatment Timepoint estimate      SE  df t.ratio p.value
 EV - irTOC1 Control   0         -0.00993 0.00433 112  -2.294 0.0237 *
 EV - irTOC1 Control   5         -0.00951 0.00433 112  -2.196 0.0301 *
 EV - irTOC1 W+OS      5         -0.01826 0.00433 112  -4.219 0.0001
***
 EV - irTOC1 Control   9         -0.01122 0.00433 112  -2.592 0.0108 *
 EV - irTOC1 W+OS      9         -0.02401 0.00433 112  -5.546 <.0001
***
 EV - irTOC1 Control   21        -0.01100 0.00433 112  -2.541 0.0124 *
 EV - irTOC1 W+OS      21        -0.02364 0.00433 112  -5.461 <.0001
***
 EV - irTOC1 Control   33        -0.01014 0.00433 112  -2.342 0.0209 *
 EV - irTOC1 W+OS      33        -0.02131 0.00433 112  -4.923 <.0001
***
 EV - irTOC1 Control   45        -0.00562 0.00433 112  -1.297 0.1973
 EV - irTOC1 W+OS      45        -0.05480 0.00433 112 -12.659 <.0001
***
 EV - irTOC1 Control   69        -0.02524 0.00433 112  -5.830 <.0001
***
 EV - irTOC1 W+OS      69        -0.05605 0.00433 112 -12.946 <.0001
***
 EV - irTOC1 W+OS      93        -0.07948 0.00433 112 -18.358 <.0001
***
```

**Nicotine vs. dicaffeoylspermidine:**

ANOVA results for normalized differences in concentration between nicotine and dicaffeoylspermidine ("Norm_Nic_DCS_conc") as response variable:

```
Response: Norm_Nic_DCS_conc
                              Df  Sum Sq Mean Sq  F value    Pr(>F)
```

```
Genotype                      1 0.61560 0.61560 767.6298 < 2.2e-16
***
Treatment                     1 0.09933 0.09933 123.8544 < 2.2e-16
***
Timepoint                     7 0.61212 0.08745 109.0417 < 2.2e-16
***
Genotype:Treatment            1 0.01056 0.01056  13.1729 0.0004295
***
Genotype:Timepoint            7 0.02848 0.00407   5.0739 4.988e-05
***
Treatment:Timepoint           5 0.12672 0.02534  31.6035 < 2.2e-16
***
Genotype:Treatment:Timepoint  5 0.03691 0.00738   9.2041 2.318e-07
***
Residuals                   112 0.08982 0.00080
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
EMMeans pairwise contrasts by treatment and time point in hours
("Treatment","Timepoint"):
contrast    Treatment Timepoint estimate     SE  df t.ratio p.value
 EV - irTOC1 Control   0          -0.0997 0.0179 112  -5.565 <.0001
***
 EV - irTOC1 Control   5          -0.0905 0.0179 112  -5.055 <.0001
***
 EV - irTOC1 W+OS      5          -0.1316 0.0179 112  -7.348 <.0001
***
 EV - irTOC1 Control   9          -0.1834 0.0179 112 -10.238 <.0001
***
 EV - irTOC1 W+OS      9          -0.0867 0.0179 112  -4.841 <.0001
***
 EV - irTOC1 Control   21         -0.1313 0.0179 112  -7.332 <.0001
***
 EV - irTOC1 W+OS      21         -0.1480 0.0179 112  -8.265 <.0001
***
 EV - irTOC1 Control   33         -0.1082 0.0179 112  -6.041 <.0001
***
 EV - irTOC1 W+OS      33         -0.0797 0.0179 112  -4.448 <.0001
***
 EV - irTOC1 Control   45         -0.0757 0.0179 112  -4.227 <.0001
***
 EV - irTOC1 W+OS      45         -0.1702 0.0179 112  -9.503 <.0001
***
 EV - irTOC1 Control   69         -0.1180 0.0179 112  -6.587 <.0001
***
 EV - irTOC1 W+OS      69         -0.2251 0.0179 112 -12.567 <.0001
***
```

```
 EV - irTOC1 W+OS      93         -0.2087 0.0179 112 -11.651 <.0001
***
```

Normalized differences in $^{15}N$ incorporation vs. nicotine (Figure **4e,f**):

**Nicotine vs. caffeoylputrescine:**

```
ANOVA results for normalized differences in incorporation rates
between nicotine and caffeoylputrescine ("Norm_Nic_CP_15N_incorp") as
response variable:
Response: Norm_Nic_CP_15N_incorp
                             Df  Sum Sq Mean Sq  F value    Pr(>F)
Genotype                      1  6.4892  6.4892 1683.518 < 2.2e-16
***
Treatment                     1  7.5818  7.5818 1966.984 < 2.2e-16
***
Timepoint                     7 10.1727  1.4532  377.023 < 2.2e-16
***
Genotype:Treatment            1  0.4213  0.4213  109.310 < 2.2e-16
***
Genotype:Timepoint            7  1.6013  0.2288   59.349 < 2.2e-16
***
Treatment:Timepoint           5  0.2299  0.0460   11.930 3.001e-09
***
Genotype:Treatment:Timepoint  5  3.1248  0.6250  162.134 < 2.2e-16
***
Residuals                   111  0.4279  0.0039
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
EMMeans pairwise contrasts by treatment and time point in hours
("Treatment","Timepoint"):
contrast    Treatment Timepoint estimate     SE  df t.ratio p.value
 EV - irTOC1 Control   0            0.000 0.0393 111   0.000 1.0000
 EV - irTOC1 Control   5            0.000 0.0393 111   0.000 1.0000
 EV - irTOC1 W+OS      5           -0.931 0.0393 111 -23.713 <.0001
***
 EV - irTOC1 Control   9           -0.999 0.0393 111 -25.446 <.0001
***
 EV - irTOC1 W+OS      9           -0.260 0.0393 111  -6.627 <.0001
***
 EV - irTOC1 Control   21          -1.088 0.0416 111 -26.123 <.0001
***
 EV - irTOC1 W+OS      21          -0.266 0.0393 111  -6.774 <.0001
***
 EV - irTOC1 Control   33          -1.092 0.0393 111 -27.798 <.0001
***
 EV - irTOC1 W+OS      33          -0.121 0.0393 111  -3.075 0.0027 **
```

```
 EV - irTOC1 Control   45          -0.412 0.0393 111 -10.489 <.0001
***
 EV - irTOC1 W+OS      45          -0.183 0.0393 111  -4.656 <.0001
***
 EV - irTOC1 Control   69          -0.286 0.0393 111  -7.293 <.0001
***
 EV - irTOC1 W+OS      69          -0.269 0.0393 111  -6.860 <.0001
***
 EV - irTOC1 W+OS      93          -0.240 0.0393 111  -6.118 <.0001
***
```

**Nicotine vs. dicaffeoylspermidine:**

ANOVA results for normalized differences in incorporation rates between nicotine and dicaffeoylspermidine (“Norm_Nic_DCS_15N_incorp”) as response variable:

```
Response: Norm_Nic_DCS_15N_incorp
                             Df Sum Sq Mean Sq  F value    Pr(>F)
Genotype                      1 3.4671  3.4671 245.6765 < 2.2e-16 ***
Treatment                     1 0.1681  0.1681  11.9133 0.0007894 ***
Timepoint                     7 3.2123  0.4589  32.5172 < 2.2e-16 ***
Genotype:Treatment            1 0.0088  0.0088   0.6252 0.4308100
Genotype:Timepoint            7 0.3125  0.0446   3.1638 0.0043447 **
Treatment:Timepoint           5 1.5519  0.3104  21.9938 2.947e-15 ***
Genotype:Treatment:Timepoint  5 0.0822  0.0164   1.1654 0.3307764
Residuals                   111 1.5665  0.0141
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in hours (“Treatment”,“Timepoint”):

```
contrast    Treatment Timepoint estimate     SE  df t.ratio p.value
 EV - irTOC1 Control   0           -0.376 0.0751 111 -5.006  <.0001
***
 EV - irTOC1 Control   5           -0.343 0.0751 111 -4.568  <.0001
***
 EV - irTOC1 W+OS      5           -0.333 0.0751 111 -4.428  <.0001
***
 EV - irTOC1 Control   9           -0.496 0.0751 111 -6.596  <.0001
***
 EV - irTOC1 W+OS      9           -0.309 0.0751 111 -4.107  0.0001
***
 EV - irTOC1 Control   21          -0.409 0.0797 111 -5.128  <.0001
***
 EV - irTOC1 W+OS      21          -0.503 0.0751 111 -6.700  <.0001
***
 EV - irTOC1 Control   33          -0.288 0.0751 111 -3.837  0.0002
***
```

```
 EV - irTOC1 W+OS      33          -0.291 0.0751 111 -3.869  0.0002
***
 EV - irTOC1 Control   45          -0.200 0.0751 111 -2.665  0.0089 **
 EV - irTOC1 W+OS      45          -0.347 0.0751 111 -4.614  <.0001
***
 EV - irTOC1 Control   69          -0.206 0.0751 111 -2.742  0.0071 **
 EV - irTOC1 W+OS      69          -0.177 0.0751 111 -2.353  0.0204 *
 EV - irTOC1 W+OS      93          -0.139 0.0751 111 -1.856  0.0661 .
```

# Figure 5:

## Outlier removal:

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

## Secondary metabolites (leaves):

**Nicotine:**

ANOVA results for nicotine concentration (“value”) as response variable:

```
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
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

---

*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
```

**Caffeoylputrescine:**

ANOVA results for caffeoylputrescine concentration (“value”) as response variable:

```
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
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
EMMeans pairwise contrasts by treatment ("Treatment"):
contrast    Treatment  estimate     SE df t.ratio p.value
 EV - irTOC1 W+OS         455726 233285 36 1.954   0.0586 .
 EV - irTOC1 W+OS + MCP   741900 233285 36 3.180   0.0030 **
```

**Dicaffeoylspermidine:**

```
ANOVA results for dicaffeoylspermidine concentration ("value") as
response variable:
Response: value
                   Df     Sum Sq    Mean Sq F value    Pr(>F)
Genotype            1 3.4405e+13 3.4405e+13 24.6086 1.694e-05 ***
Treatment           1 2.0811e+12 2.0811e+12  1.4886   0.23037
Genotype:Treatment  1 8.6750e+12 8.6750e+12  6.2049   0.01749 *
Residuals          36 5.0331e+13 1.3981e+12
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
EMMeans pairwise contrasts by by treatment ("Treatment"):
contrast    Treatment  estimate     SE df t.ratio p.value
 EV - irTOC1 W+OS         923458 528788 36 1.746   0.0893 .
 EV - irTOC1 W+OS + MCP  2786248 528788 36 5.269   <.0001 ***
```

Secondary metabolites (roots):

**Nicotine:**

```
ANOVA results for nicotine concentration ("value") as response
variable:
Response: value
                   Df     Sum Sq    Mean Sq F value   Pr(>F)
Genotype            1 2.2261e+13 2.2261e+13 13.2554 0.002201 **
Treatment           1 1.3004e+13 1.3004e+13  7.7435 0.013307 *
Genotype:Treatment  1 2.6368e+07 2.6368e+07  0.0000 0.996887
Residuals          16 2.6870e+13 1.6794e+12
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
EMMeans pairwise contrasts by treatment ("Treatment"):
contrast    Treatment  estimate     SE df t.ratio p.value
 EV - irTOC1 W+OS       -2112308 819601 16 -2.577  0.0203 *
 EV - irTOC1 W+OS + MCP -2107715 819601 16 -2.572  0.0205 *
```

**Caffeoylputrescine:**

```
ANOVA results for caffeoylputrescine concentration ("value") as
response variable:
Response: value
```

```
                    Df     Sum Sq    Mean Sq F value Pr(>F)
Genotype             1 3.9958e+09 3995812901  1.0884 0.3123
Treatment            1 7.2446e+09 7244554470  1.9733 0.1792
Genotype:Treatment   1 4.3849e+09 4384898597  1.1944 0.2906
Residuals           16 5.8740e+10 3671279922
---------------------------------------------
EMMeans pairwise contrasts by treatment ("Treatment"):
contrast    Treatment  estimate    SE df t.ratio p.value
 EV - irTOC1 W+OS         -57883 38321 16 -1.510  0.1504
 EV - irTOC1 W+OS + MCP     1344 38321 16  0.035  0.9724
```

**Dicaffeoylspermidine:**

```
ANOVA results for dicaffeoylspermidine concentration ("value") as
response variable:
Response: value
                    Df     Sum Sq   Mean Sq F value Pr(>F)
Genotype             1 2.4105e+08 241048267  0.3080 0.5866
Treatment            1 9.7492e+08 974923941  1.2456 0.2809
Genotype:Treatment   1 4.6024e+08 460238585  0.5880 0.4543
Residuals           16 1.2523e+10 782665939
---------------------------------------------
EMMeans pairwise contrasts by treatment ("Treatment"):
contrast    Treatment  estimate    SE df t.ratio p.value
 EV - irTOC1 W+OS         -16537 17694 16 -0.935  0.3639
 EV - irTOC1 W+OS + MCP     2651 17694 16  0.150  0.8828
```

## Figure 6:

### Outlier removal:

One plant identified using our outlier procedure with divergent values across a range of phenotypes was removed (EV control, replicate 1). Overall lower replicate numbers, particularly for biomass and secondary metabolite data and later time points of fitness correlate data, were caused by an outbreak of a *Fusarium/Alternaria* pathosystem infection during the second half of the field season.

### Secondary metabolites:

**Nicotine:**

ANOVA results for nicotine concentration Peak area/g FW (Nicotine) as response variable:

```
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
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

---

Pairwise t-tests with pooled SD by Genotype (no *EMMeans* were calculated due to lack of a significant interaction factor):

```
   EE      ET
ET 0.04 *  -
TT 0.04 *  0.79

P value adjustment method: holm
```

**Caffeoylputrescine:**

ANOVA results for caffeoylputrescine concentration Peak area/g FW (CP) as response variable:

```
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
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

---

Pairwise t tests with pooled SD by Treatment (no *EMMeans* were calculated due to lack of a significant interaction factor):

```
     Control
W+OS 0.029 *

P value adjustment method: holm
```

**Dicaffeoylspermidine:**

```
ANOVA results for dicaffeoylspermidine concentration Peak area/g FW
(DCS) as response variable:
Response: DCS
                    Df     Sum Sq    Mean Sq F value  Pr(>F)
Genotype             2 6.0307e+11 3.0153e+11  3.9294 0.03282 *
Treatment            1 1.1942e+10 1.1942e+10  0.1556 0.69656
Genotype:Treatment   2 2.9374e+09 1.4687e+09  0.0191 0.98106
Residuals           25 1.9184e+12 7.6737e+10
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
Pairwise t tests with pooled SD by Genotype (no EMMeans were
calculated due to lack of a significant interaction factor):

   EE       ET
ET 0.028 *  -
TT 0.487    0.087 .

P value adjustment method: holm
```

Reproductive correlates:

**Seed capsules**

ANOVA results for seed capsules (Capsules) as response variable:

```
Response: Capsules
                            Df  Sum Sq Mean Sq F value  Pr(>F)
Genotype                     2  199.22  99.610 45.7673 < 2e-16 ***
Treatment                    1    8.34   8.336  3.8299 0.05056 .
Time                        19 1398.75  73.618 33.8250 < 2e-16 ***
Genotype:Treatment           2   12.93   6.466  2.9708 0.05160 .
Genotype:Time               38  409.37  10.773  4.9498 < 2e-16 ***
Treatment:Time              19   67.80   3.568  1.6395 0.04051 *
Genotype:Treatment:Time     38   55.07   1.449  0.6659 0.94133
Residuals                 1320 2872.91   2.176
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

---

*EMMeans* pairwise contrasts by genotype and time point in days ("Genotype","Time"):

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

```
contrast          Genotype Time      estimate    SE   df t.ratio
p.value
 Control - W+OS    EE       30          0.0000 0.616 1320  0.000
1.0000
 Control - W+OS    ET       30          0.0769 0.591 1320  0.130
0.8964
 Control - W+OS    TT       30          0.0000 0.602 1320  0.000
1.0000
 Control - W+OS    EE       31         -0.3333 0.616 1320 -0.541
0.5884
 Control - W+OS    ET       31         -0.0192 0.591 1320 -0.033
0.9740
 Control - W+OS    TT       31          0.0000 0.602 1320  0.000
1.0000
 Control - W+OS    EE       32         -0.0606 0.616 1320 -0.098
0.9216
 Control - W+OS    ET       32          0.1282 0.591 1320  0.217
0.8282
 Control - W+OS    TT       32          0.0000 0.602 1320  0.000
1.0000
 Control - W+OS    EE       33         -0.1288 0.616 1320 -0.209
0.8344
 Control - W+OS    ET       33          0.0833 0.591 1320  0.141
0.8878
 Control - W+OS    TT       33          0.0000 0.602 1320  0.000
1.0000
 Control - W+OS    EE       34         -0.1288 0.616 1320 -0.209
0.8344
```

```
 Control - W+OS    ET       34          0.0833 0.591 1320  0.141
0.8878
 Control - W+OS    TT       34          0.0000 0.602 1320  0.000
1.0000
 Control - W+OS    EE       35         -0.1288 0.616 1320 -0.209
0.8344
 Control - W+OS    ET       35          0.0833 0.591 1320  0.141
0.8878
 Control - W+OS    TT       35          0.0000 0.602 1320  0.000
1.0000
 Control - W+OS    EE       36         -0.0909 0.616 1320 -0.148
0.8827
 Control - W+OS    ET       36          0.9295 0.591 1320  1.574
0.1158
 Control - W+OS    TT       36          0.0000 0.602 1320  0.000
1.0000
 Control - W+OS    EE       37         -0.0909 0.616 1320 -0.148
0.8827
 Control - W+OS    ET       37          0.9295 0.591 1320  1.574
0.1158
 Control - W+OS    TT       37          0.0000 0.602 1320  0.000
1.0000
 Control - W+OS    EE       38         -0.0909 0.616 1320 -0.148
0.8827
 Control - W+OS    ET       38          0.9295 0.591 1320  1.574
0.1158
 Control - W+OS    TT       38          0.0000 0.602 1320  0.000
1.0000
 Control - W+OS    EE       39         -0.0909 0.616 1320 -0.148
0.8827
 Control - W+OS    ET       39          0.9295 0.591 1320  1.574
0.1158
 Control - W+OS    TT       39          0.0000 0.602 1320  0.000
1.0000
 Control - W+OS    EE       40          1.7273 0.616 1320  2.805
0.0051 **
 Control - W+OS    ET       40          4.0833 0.591 1320  6.914
<.0001 ***
 Control - W+OS    TT       40          0.0000 0.602 1320  0.000
1.0000
----------------------------------------------
```

*EMMeans* pairwise contrasts by treatment and time point in days (“Treatment”,“Time”):

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

```
contrast Treat.  Time      estimate    SE   df t.ratio p.value
 EE - ET  Control 30         -0.0769 0.604 1320 -0.127  0.9911
 EE - TT  Control 30          0.0000 0.616 1320  0.000  1.0000
 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 | *** |

```
 EE - ET  W+OS    38          0.0833 0.602 1320  0.138  0.9895
 EE - TT  W+OS    38          1.9167 0.602 1320  3.182  0.0043 **
 ET - TT  W+OS    38          1.8333 0.602 1320  3.044  0.0067 **
 EE - ET  Control 39         -0.9371 0.604 1320 -1.550  0.2678
 EE - TT  Control 39          1.8258 0.616 1320  2.965  0.0087 **
 ET - TT  Control 39          2.7628 0.591 1320  4.678  <.0001 ***
 EE - ET  W+OS    39          0.0833 0.602 1320  0.138  0.9895
 EE - TT  W+OS    39          1.9167 0.602 1320  3.182  0.0043 **
 ET - TT  W+OS    39          1.8333 0.602 1320  3.044  0.0067 **
 EE - ET  Control 40         -2.2727 0.604 1320 -3.760  0.0005 ***
 EE - TT  Control 40          4.3106 0.616 1320  7.000  <.0001 ***
 ET - TT  Control 40          6.5833 0.591 1320 11.147  <.0001 ***
 EE - ET  W+OS    40          0.0833 0.602 1320  0.138  0.9895
 EE - TT  W+OS    40          2.5833 0.602 1320  4.289  0.0001 ***
 ET - TT  W+OS    40          2.5000 0.602 1320  4.151  0.0001 ***

P value adjustment: tukey method for comparing a family of 3 estimates
---------------------------------------------
EM Trends pairwise contrasts with seed capsules against time in days
("Time.trend"):
$emtrends
 Treat.  Genotype Time.trend     SE   df lower.CL upper.CL
 Control EE           0.1700 0.0187 1428   0.1332    0.207
 W+OS    EE           0.1516 0.0179 1428   0.1164    0.187
 Control ET           0.2511 0.0172 1428   0.2172    0.285
 W+OS    ET           0.1521 0.0179 1428   0.1169    0.187
 Control TT           0.0237 0.0179 1428  -0.0115    0.059 .
 W+OS    TT           0.0237 0.0179 1428  -0.0115    0.059 .

Confidence level used: 0.95

$contrasts
 contrast                 estimate     SE   df t.ratio p.value
 Control,EE - W+OS   ,EE  0.018427 0.0260 1428  0.710  0.9808
 Control,EE - Control,ET -0.081077 0.0255 1428 -3.183  0.0186 *
 Control,EE - W+OS   ,ET  0.017926 0.0260 1428  0.691  0.9830
 Control,EE - Control,TT  0.146246 0.0260 1428  5.635  <.0001 ***
 Control,EE - W+OS   ,TT  0.146246 0.0260 1428  5.635  <.0001 ***
 W+OS   ,EE - Control,ET -0.099504 0.0249 1428 -3.998  0.0009 ***
 W+OS   ,EE - W+OS   ,ET -0.000501 0.0254 1428 -0.020  1.0000
 W+OS   ,EE - Control,TT  0.127820 0.0254 1428  5.036  <.0001 ***
 W+OS   ,EE - W+OS   ,TT  0.127820 0.0254 1428  5.036  <.0001 ***
 Control,ET - W+OS   ,ET  0.099002 0.0249 1428  3.978  0.0010 ***
 Control,ET - Control,TT  0.227323 0.0249 1428  9.133  <.0001 ***
 Control,ET - W+OS   ,TT  0.227323 0.0249 1428  9.133  <.0001 ***
 W+OS   ,ET - Control,TT  0.128321 0.0254 1428  5.056  <.0001 ***
 W+OS   ,ET - W+OS   ,TT  0.128321 0.0254 1428  5.056  <.0001 ***
 Control,TT - W+OS   ,TT  0.000000 0.0254 1428  0.000  1.0000
```

P value adjustment: tukey method for comparing a family of 6 estimates

**Flowers**

ANOVA results for flowers (Flowers) as response variable:

```
Response: Flowers
                          Df  Sum Sq Mean Sq F value    Pr(>F)
Genotype                   2   890.8   445.4  5.1315  0.006567 **
Treatment                  1   118.2   118.2  1.3617  0.244393
Time                       7 29722.4  4246.1 48.9176 < 2.2e-16 ***
Genotype:Treatment         2    11.2     5.6  0.0647  0.937331
Genotype:Time             14  2255.7   161.1  1.8562  0.031829 *
Treatment:Time             7   274.1    39.2  0.4511  0.868902
Genotype:Treatment:Time   13   637.1    49.0  0.5646  0.880703
Residuals                242 21005.6    86.8
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
```

*EMMeans* pairwise contrasts by genotype and time point in days ("Genotype","Time"):

```
contrast          Genotype Timepoint estimate    SE  df t.ratio
p.value
 Control - W+OS    EE       21         -0.3333  8.50 242 -0.039
0.9688
 Control - W+OS    ET       21         -0.1667  7.12 242 -0.023
0.9813
 Control - W+OS    TT       21          0.0000  8.50 242  0.000
1.0000
 Control - W+OS    EE       24         -1.3333  7.61 242 -0.175
0.8610
 Control - W+OS    ET       24          0.0000  7.61 242  0.000
1.0000
 Control - W+OS    TT       24          0.0000  7.61 242  0.000
1.0000
 Control - W+OS    EE       25         -1.2000  4.17 242 -0.288
0.7736
 Control - W+OS    ET       25          0.2756  3.73 242  0.074
0.9411
 Control - W+OS    TT       25          0.0909  3.89 242  0.023
0.9814
 Control - W+OS    EE       28         -1.1444  4.28 242 -0.267
0.7894
 Control - W+OS    ET       28          0.4336  3.82 242  0.114
0.9097
 Control - W+OS    TT       28         -0.1818  3.97 242 -0.046
0.9635
```

```
 Control - W+OS    EE       31         -0.9028  4.53 242 -0.199
0.8421
 Control - W+OS    ET       31         -0.1111  4.70 242 -0.024
0.9811
 Control - W+OS    TT       31         -0.8611  4.53 242 -0.190
0.8493
 Control - W+OS    EE       35          nonEst    NA  NA     NA
NA (note: due to human error, only one EE plant was measured on day
35)
 Control - W+OS    ET       35         10.0000  8.50 242  1.176
0.2408
 Control - W+OS    TT       35        -15.3333 10.76 242 -1.425
0.1554
 Control - W+OS    EE       36          0.2619  5.18 242  0.051
0.9597
 Control - W+OS    ET       36         -0.8333  5.60 242 -0.149
0.8818
 Control - W+OS    TT       36          5.2540  4.70 242  1.119
0.2642
 Control - W+OS    EE       40         15.6667  7.12 242  2.202
0.0286 *
 Control - W+OS    ET       40          9.7500 10.42 242  0.936
0.3502
 Control - W+OS    TT       40         -1.8333  7.12 242 -0.258
0.7969
---------------------------------------------
EM Trends pairwise contrasts with flowers against time ("DCS.trend"):
$emtrends
 Treat.  Genotype Time.trend    SE  df lower.CL upper.CL
 Control EE            2.37 0.310 277    1.763     2.98
 W+OS    EE            1.58 0.317 277    0.960     2.21
 Control ET            1.83 0.272 277    1.296     2.37
 W+OS    ET            1.57 0.359 277    0.861     2.27
 Control TT            1.35 0.303 277    0.750     1.94
 W+OS    TT            1.18 0.289 277    0.615     1.75

Confidence level used: 0.95

$contrasts
 contrast                estimate    SE  df t.ratio p.value
 Control,EE - W+OS   ,EE   0.7884 0.443 277  1.778  0.4816
 Control,EE - Control,ET   0.5410 0.412 277  1.312  0.7784
 Control,EE - W+OS   ,ET   0.8055 0.474 277  1.700  0.5333
 Control,EE - Control,TT   1.0255 0.434 277  2.365  0.1723
 Control,EE - W+OS   ,TT   1.1891 0.424 277  2.807  0.0593 .
 W+OS   ,EE - Control,ET  -0.2475 0.418 277 -0.592  0.9915
 W+OS   ,EE - W+OS   ,ET   0.0171 0.479 277  0.036  1.0000
 W+OS   ,EE - Control,TT   0.2371 0.439 277  0.540  0.9945
```

```
 W+OS   ,EE - W+OS   ,TT   0.4007 0.429 277  0.934  0.9375
 Control,ET - W+OS   ,ET   0.2646 0.450 277  0.587  0.9918
 Control,ET - Control,TT   0.4846 0.408 277  1.189  0.8421
 Control,ET - W+OS   ,TT   0.6482 0.397 277  1.632  0.5778
 W+OS   ,ET - Control,TT   0.2200 0.470 277  0.468  0.9972
 W+OS   ,ET - W+OS   ,TT   0.3836 0.461 277  0.833  0.9612
 Control,TT - W+OS   ,TT   0.1636 0.419 277  0.390  0.9988

P value adjustment: tukey method for comparing a family of 6 estimates
```

Biomass:

**Shoots**

```
ANOVA results for shoot biomass in grams (Shoot) as response variable:
Response: Shoot
                Df  Sum Sq Mean Sq F value  Pr(>F)
Genotype         2  126.49  63.243  1.1273 0.33540
Treat.           1    3.41   3.406  0.0607 0.80681
Genotype:Treat.  2  539.99 269.995  4.8124 0.01424 *
Residuals       35 1963.62  56.103
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
EMMeans pairwise contrasts by treatment ("Treat"):
contrast Treat    estimate   SE df t.ratio p.value
 EE - ET  Control    -1.52 4.69 35 -0.324  0.9439
 EE - TT  Control   -12.26 4.00 35 -3.063  0.0114 *
 ET - TT  Control   -10.74 4.69 35 -2.288  0.0708 .
 EE - ET  W+OS       -3.51 4.93 35 -0.712  0.7582
 EE - TT  W+OS        2.04 3.35 35  0.610  0.8155
 ET - TT  W+OS        5.55 4.93 35  1.126  0.5045

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

**Roots**

```
ANOVA results for root biomass in grams (Root) as response variable:
Response: Root
                Df  Sum Sq Mean Sq F value   Pr(>F)
Genotype         2  0.5482  0.2741  0.5002 0.610693
Treat.           1  0.2854  0.2854  0.5208 0.475287
Genotype:Treat.  2  8.6037  4.3019  7.8503 0.001526 **
Residuals       35 19.1795  0.5480
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
EMMeans pairwise contrasts by treatment ("Treat"):
contrast Treat    estimate    SE df t.ratio p.value
```

```
 EE - ET  Control  -0.0886 0.464 35 -0.191  0.9801
 EE - TT  Control  -1.2921 0.396 35 -3.266  0.0067 **
 ET - TT  Control  -1.2035 0.464 35 -2.594  0.0358 *
 EE - ET  W+OS     -0.4419 0.487 35 -0.907  0.6396
 EE - TT  W+OS      0.4754 0.331 35  1.436  0.3339
 ET - TT  W+OS      0.9173 0.487 35  1.882  0.1588

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

# Figure S2:

## Outlier removal:

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

## Ethylene burst:

ANOVA results for ethylene abundance in nL/gFW (“Ethylene”) as response variable:

```
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
----------
```

*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
```

```
 266/205 - 266/212 W+Ms       12.0601 4.12 49  2.924  0.0398 *
 266/205 - 205/205 W+Ms        9.4074 4.12 49  2.281  0.1685
 266/205 - 212/212 W+Ms       12.1414 4.12 49  2.944  0.0379 *
 266/212 - 205/205 W+Ms       -2.6527 4.12 49 -0.643  0.9671
 266/212 - 212/212 W+Ms        0.0813 4.12 49  0.020  1.0000
 205/205 - 212/212 W+Ms        2.7340 4.12 49  0.663  0.9633

P value adjustment: tukey method for comparing a family of 5 estimates
```

## Figure S3:

Outlier removal:

No outliers were removed from this dataset.

Transcript abundance:

**ACS3:**

```
ANOVA results for transcript abundance (“Normalized”) as response
variable:
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
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
---------------------------------------------
Pairwise t tests with pooled SD by Treatment (no EMMeans were
calculated due to lack of a significant interaction factor):

        Control
W+OS    3.8e-14 ***

P value adjustment method: holm
```

**ACO:**

```
ANOVA results for transcript abundance (“Normalized”) as response
variable:
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
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
---------------------------------------------
Pairwise t tests with pooled SD by Treatment (no EMMeans were
calculated due to lack of a significant interaction factor):

        Control
W+OS    <2e-16 ***

P value adjustment method: holm
```

**ETR1:**

```
ANOVA results for transcript abundance (“Normalized”) as response
variable:
Response: Normalized
                   Df   Sum Sq  Mean Sq F value   Pr(>F)
Treatment           1 0.058560 0.058560  8.3205 0.008605 **
Genotype            1 0.006604 0.006604  0.9383 0.343234
Treatment:Genotype  1 0.005808 0.005808  0.8253 0.373481
Residuals          22 0.154837 0.007038
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
----------------------------------------------
Pairwise t tests with pooled SD by Treatment (no EMMeans were
calculated due to lack of a significant interaction factor):

        Control
W+OS    0.0079 **

P value adjustment method: holm
```

**CTR1:**

```
ANOVA results for transcript abundance (“Normalized”) as response
variable:
Response: Normalized
                   Df   Sum Sq  Mean Sq F value    Pr(>F)
Treatment           1 0.078012 0.078012 78.1985 1.076e-08 ***
Genotype            1 0.000324 0.000324  0.3248    0.5745
Treatment:Genotype  1 0.000497 0.000497  0.4980    0.4878
Residuals          22 0.021947 0.000998
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
----------------------------------------------
Pairwise t tests with pooled SD by Treatment (no EMMeans were
calculated due to lack of a significant interaction factor):

        Control
W+OS    3.2e-09 ***

P value adjustment method: holm
```

**EIN2:**

```
ANOVA results for transcript abundance (“Normalized”) as response
variable:
Response: Normalized
                   Df  Sum Sq  Mean Sq F value Pr(>F)
Treatment           1 0.01717 0.017172  0.6242 0.4379
Genotype            1 0.01742 0.017422  0.6333 0.4347
Treatment:Genotype  1 0.03024 0.030241  1.0992 0.3058
Residuals          22 0.60525 0.027511
```

**EIN3:**

```
ANOVA results for transcript abundance (“Normalized”) as response
variable:
Response: Normalized
                   Df    Sum Sq   Mean Sq F value  Pr(>F)
Treatment           1 0.0059295 0.0059295  7.7812 0.01069 *
Genotype            1 0.0006521 0.0006521  0.8558 0.36496
Treatment:Genotype  1 0.0012192 0.0012192  1.5999 0.21915
Residuals          22 0.0167647 0.0007620
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
----------------------------------------------
Pairwise t tests with pooled SD by Treatment (no EMMeans were
calculated due to lack of a significant interaction factor):

        Control
W+OS    0.011 *

P value adjustment method: holm
```

**ERF189:**

```
ANOVA results for transcript abundance (“Normalized”) as response
variable:
Response: Normalized
                   Df     Sum Sq    Mean Sq F value   Pr(>F)
Treatment           1 1.7289e-09 1.7289e-09  9.9080 0.005872 **
Genotype            1 2.8620e-11 2.8620e-11  0.1640 0.690553
Treatment:Genotype  1 8.5890e-11 8.5890e-11  0.4923 0.492417
Residuals          17 2.9664e-09 1.7449e-10
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
----------------------------------------------
Pairwise t tests with pooled SD by Treatment (no EMMeans were
calculated due to lack of a significant interaction factor):

        Control
W+OS    0.0041 **

P value adjustment method: holm
```

## Figure S4:

### Outlier removal:

4 seedlings (EV-0uM, 205-0uM, etr1-0uM, and EV-10uM) were removed from the analysis because of delayed germination time.

### Hypocotyl length:

ANOVA results for hypocotyl length ("Length") as response variable:

```
Response: Length
                    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
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
----------------------------------------------
```

*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 |

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

## Figure S5:

### Outlier removal:

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

### Secondary metabolites:

**Chlorogenic acid (a):**

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

```
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
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
---------------------------------------------
EMMeans pairwise contrasts by time point in hours ("Time"):
contrast       Time estimate    SE df t.ratio p.value
 EV - irTOC1    0       1.895 0.819 30  2.313  0.0692 .
 EV - irZTL     0       2.423 0.819 30  2.958  0.0160 *
 irTOC1 - irZTL 0       0.528 0.819 30  0.645  0.7968
 EV - irTOC1    72      1.972 0.819 30  2.407  0.0568 .
 EV - irZTL     72      1.672 0.819 30  2.041  0.1199
 irTOC1 - irZTL 72     -0.300 0.819 30 -0.366  0.9290

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

**Chlorogenic acid (b):**

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

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

```
 EE - ET  cm         -356258 269595 24 -1.321  0.3974
 EE - TT  cm          315481 269595 24  1.170  0.4819
 ET - TT  cm          671738 257049 24  2.613  0.0390 *

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