New Phytologist Supporting Information Figs S1-S9, Tables S1-S2 and Methods S1

**Article title:** Mechanisms of glacial-to-future atmospheric CO₂ effects on plant immunity

**Authors:** Alex Williams, Pierre Pétriacq, Roland E. Schwarzenbacher, David J. Beerling & Jurriaan Ton

**Article acceptance date:** 26 December 2017
Fig. S1 Effects of CO₂ on plant development. Data represent average leaf numbers (± SE; n = 8) plotted against time (days) at ambient CO₂ (aCO₂; 400 ppm; dashed line), subambient CO₂ (saCO₂; 200 ppm; dotted line) and elevated CO₂ (eCO₂; 1200 ppm; straight line). Inserts show typical rosette sizes of 4.5-week old plants. Red and blue lines illustrate differences in absolute age at the 8- and 18-leaf stage, respectively. Shown are results from a representative experiment that was repeated twice.
Fig. S2 Representative examples of the four different Hpa colonization classes that were used to quantify Arabidopsis resistance. To visualise Hpa colonisation, leaves were stained with lactophenol trypan-blue, as described previously (Luna et al. 2012). Class I is defined by a lack of hyphal growth; Class II sustains hyphal development, but not the production of asexual conidiospores; Class III is characterised by extensive hyphal colonisation and the formation of conidiophores and asexual conidiospores; Class IV is similar as class III, but with additional formation of sexual oospores (> 10 per leaf). Black bars indicate scales.
Fig. S3 qPCR-based quantification of pathogen biomass to confirm the development-independent effects of CO₂ on resistance against *Hpa* and *Pc*. (a) Relative quantification of *Hpa* DNA was based on the *Hpa* actin gene (ID: 807716). (b) Relative quantification of *Pc* DNA was based on the *Pc* β-tubulin gene. Data represent relative DNA quantities normalized to Arabidopsis *ACT2* (*At3g18780*) (± SD, n = 4). Letters indicate statistical differences (ANOVA + Tukey post-hoc analysis; *P* < 0.05). For details about DC and timing of pathogen inoculation, see legend to Fig. 1.
Fig. S4 SA signalling in saCO₂-induced resistance against *Hpa*. (a) Levels of SA-inducible *PR1* gene expression in 8-leaf Col-0 at saCO₂ (200 ppm) and aCO₂ (400 ppm). Shown are box plots of relative transcript values (n = 3; means are indicated by X) at 24 hours after treatment. (b) Quantification of *Hpa* resistance at saCO₂ and aCO₂ in Col-0, the SA insensitive *npr1-1* mutant, and the SA production mutant *sid2-1* at the 8-leaf stage. Shown are relative numbers of leaves (n > 50) in *Hpa* colonization classes of increasing severity (I–IV) at 7 dpi. Letters - (a) ANOVA with Tukey HSD post hoc analysis - or asterisks - (b) Fisher’s exact test - indicate statistically significant differences between conditions (*P* < 0.05). The pathogenicity assays with *sid2-1* and *npr1-1* were repeated with similar results.
Fig. S5 Global metabolic signatures of mock- and *Hpa*-inoculated Arabidopsis (Col-0) at the 8-leaf growth stage at *saCO₂* (200 ppm) and *aCO₂* (400 ppm). Shown are principal component analysis (PCA) plots of negative (ESI⁻; 4497 ions) and positive (ESI⁺; 5683 ions) ionizations, obtained by UPLC-Q-TOF analysis of methanol extracts from leaf tissue at 24 and 72 hpi. Samples from plants grown at *saCO₂* are indicated by circles; samples from plants at *aCO₂* are indicated by squares. Black/blue symbols indicate mock-inoculated plants; grey/ light blue symbols indicate samples from *Hpa*-inoculated plants.
(a)

UPLC-Q-TOF

\[ \downarrow \]

XCMS peak alignment

\[ \downarrow \]

ESI-: 4,479 ions \hspace{1cm} ESI+: 5,683 ions

\[ \downarrow \]

ANOVA + FDR \hspace{1cm} ANOVA + FDR

\[ \downarrow \]

266 ions (ESI- and ESI+)

\[ \downarrow \]

2-way ANOVA for 24 hpi \hspace{1cm} 2-way ANOVA for 72 hpi

\[ \downarrow \]

Pearson's correlation cluster analysis of ions that are statistically influenced by CO\(_2\), Hpa, or CO\(_2\) x Hpa

\[ \downarrow \]

selection of ion clusters that are induced or primed for Hpa-induced accumulation by saCO\(_2\)

---

(b)

| Factor     | 24 hpi | 72 hpi |
|------------|--------|--------|
| CO\(_2\)   | 111    | 57     |
| Hpa        | 65     | 34     |
| CO\(_2\) x Hpa | 24    | 17     |
Fig S6 Selection of ions that are induced or primed for Hpa-induced accumulation by saCO₂. (a) Schematic pipeline of the selection procedure. (b) Numbers of statistically significant ions by 2-way ANOVA of the selection of 266 ions. (c) Hierarchical cluster analysis (Pearson’s correlation) of ions that are significantly influenced by CO₂, Hpa or the interaction CO₂ x Hpa. Highlighted are ion clusters showing direct induction (saCO₂ - induced) or priming for Hpa-induced accumulation by saCO₂ (saCO₂ - primed).
Fig. S7 Extracellular H$_2$O$_2$ in saCO$_2$-induced resistance against Hpa. (a) Visualization of extracellular H$_2$O$_2$ accumulation in leaves of Arabidopsis (Col-0) at aCO$_2$ (400 ppm) and saCO$_2$ (200 ppm). Shown are 3,3’-diaminobenzidine (DAB)-stained leaves at 48 hours after mock (water) or Hpa inoculation. Bar = 1 mm. (b) Quantification of DAB staining signal by image analysis. Shown are mean values of the stained proportion of the leaf area (± SD, n = 5). (c) Evaluation of Hpa resistance at aCO$_2$ and saCO$_2$ in Col-0 and rbohD/F plants at the 8-leaf stage. The rbohD/F double mutant is impaired in production of extracellular H$_2$O$_2$ by NADP-dependent oxidase. Shown are relative numbers of leaves (n > 50) in Hpa colonization classes of increasing severity (I–IV) at 7 dpi. Asterisks indicate statistically significant differences between CO$_2$ conditions (Fisher’s exact test; P < 0.05).
Fig. S8. Selection of gox1-2 (SALK_051930) and haox1-2 (SALK_022285) knock-down mutants. (a) PCR confirmation of homozygous T-DNA insertions. Gene models show locations of T-DNA insertions in promoter regions of GOX1 and HAOX1. Images show PCR products from 1) mutant DNA with LP + RP primers (no band); 2) Col-0 DNA with LP + RP primers, and 3), mutant DNA with LBb1.3 + RP primers. (b) Impacts of knock-down mutations on transcription of GOX1 and HAOX1 in gox1-2 and haox1-2 plants, respectively. Shown are mean values of relative transcript levels (± SD; n = 5) in shoot tissues of 3-week old plants. Asterisks indicate statistically significant reductions in relative transcript level compared to wild-type plants (Col-0; Student’s t-test, \( P < 0.05 \)). The experiment was repeated with similar results. (c) Growth phenotypes of 3-week old Col-0, gox1-2 and haox1-2 at aCO2 and saCO2 conditions.
Fig. S9. Impacts of Hpa inoculation on CAT2 gene expression in 8-leaf Col-0 plants at saCO$_2$ (200 ppm) and aCO$_2$ (400 ppm). Shown are mean values of relative transcript abundance (± SD, n = 5) at different hours post mock (water) or Hpa inoculation.
Table S1. Primers used for mutant genotyping and RT-qPCR analysis of gene expression

| Gene     | Gene locus | Forward primer sequence (5' - 3') | Reverse primer sequence (5' - 3') |
|----------|------------|----------------------------------|----------------------------------|
| **Genotyping** |            |                                  |                                  |
| GOX1     | AT3G14420  | CCG AAA GCT ATT AAA CAG CCC      | CTG ACA TGG CAC CCA ACT TCC      |
| HAOX1    | AT3G14130  | GCA GAA TGG AGG GGT TTA GTC      | CAT GCA AGA ATC TTG CTC CTC      |
| SALK Insert (LBb1.3) |          | ATT TTG CCG ATT TCG GAA C        |                                  |
| **qPCR primers** |          |                                  |                                  |
| GOX1     | AT3G14420  | AGA ACA GCA GCA ACA CAG AAC      | CAC TAG GCT TGG TTT GTG ATC TGA TA |
| HAOX1    | AT3G14130  | GAA TTA AAT CTA TGC TCT GAT CCT AAA ACC | GAA CAA GTC CAA CGT ACT ATT GTC TT |
| CAT2     | AT4G35090  | CGA GGT ATG ACC AGG TTT GTA      | CTT CCA GGC TCC TGG AAG TTG      |
| PRI      | AT2G14610  | GTG TCC GCC GTG AAG ATG T        | CGT GGT GGC AGC GTA GGT GT        |
| VSP2     | AT5G24770  | GGA CTT GCC CTA AAG AAC GAC ACC  | GTC GGT CTT CTC TGG TCC GTA TCC |
| UBC 9    | AT5G25760  | TCA CAA TTT CCA AG GTG CTG C     | TCA CTT GGG TTT GGA TCC GT        |
| SAND     | AT2G28390  | AAC TCT ATG CAG CAT T           | GGT GGT ACT AGC ACA A             |
| **DNA quantification** |            |                                  |                                  |
| HpaACT   | ID807716   | GTG TCG CAC ACT GTA CCC ATT TAT  | ATC TTC ATC ATG TAG TCG AAG T     |
| Pej-Tub   |            | CCA GTA TGT TCC CCG AGC CTG      | GAA GAG CTG ACC GAA GGG ACC       |
| AvACT2   | AT3G18780  | AAT CAC AGC ACT TGC ACC A        | GMG GGA AGC AAG AAT GGA AC        |
| sample  | retention time (min) | Adducts | Predicted mass (Da) | Error (ppm) | Putative compound | Predicted formula | Pathways |
|---------|---------------------|---------|---------------------|-------------|-------------------|------------------|----------|
| 1.2E-03 | 351.992             | [M+K]+  | 313.021             | 19          | Ludistomin H      | C15H12BrN3       | Alkaloids |
| 6.0E-03 | 422.166             | [M-H]+  | 423.168             | 13          | N-Methyl-2,3,7,8-tetramethoxy-5,6-dihydroxybenzenenitrilide-6-ethanoic acid | C24H25NO6 | Alkaloids |
| 6.6E-03 | 482.067             | [M+Cl]  | 447.108             | 22          | Pigment A aglycone | C25H19O8 | Anthocyanins |
| 1.0E-03 | 244.027             | [M+K-2H]+| 207.081             | 11          | Anthocyanidins    | C15H11O10 | Anthocyanins |
| 2.2E-04 | 933.074             | [M-H]+  | 934.071             | 10          | Vescalagin        | C41H26O26 | Anthocyanins |
| 2.2E-04 | 391.066             | [M+Na-2H]+| 370.096             | 4           | 5-Hydroxy-6-methoxycoumarin 7-glucoside | C16H10O10 | Coumarin |
| 2.2E-04 | 457.133             | [M-H]+  | 458.142             | 5           | cis-p-Coumaric acid 4-(apioyl-1->2)-glucoside | C20H12O2 | Coumarin |
| 5.3E-04 | 427.124             | [M+Na]+ | 404.126             | 20          | Calomelanol C     | C24H20O6 | Flavonoids |
| 1.3E-04 | 329.088             | [M+Na-2H]+| 284.032             | 13          | 7,4',5'-Trihydroxy-5,2'-oxido-4-phenylcoumarin | C15H10O6 | Flavonoids |
| 6.0E-04 | 639.161             | [M-H]+  | 640.164             | 6           | Laricitrin 3-rutinoside | C28H32O17 | Flavonoids |
| 9.0E-04 | 932.246             | [M-H]+  | 933.266             | 14          | Pelargonidin 3-O-[B-D-Glucopyranosyl(1->2)-[4-hydroxy-3-methoxy-E]-cinnamoyl(1->6)-B-D-glucopyranosyl(1->6)-B-D-glucopyranoside | C43H49O23 | Flavonoids |
| 2.5E-04 | 421.163             | [M-H]+  | 422.173             | 6           | Iuchromosome b10 | C25H26O6 | Flavonoids |
| 5.6E-04 | 487.123             | [M-H]+  | 488.132             | 3           | Acacetin 7-(2'-(acetylglucoside) | C24H24O11 | Flavonoids |
| 1.4E-04 | 667.080             | [M+Na-2H]+| 622.117             | 12          | Apigenin 7-glucuronosyl(1->2)-glucoside | C27H26O17 | Flavonoids |
| 3.4E-04 | 603.292             | [M-H]+  | 604.288             | 10          | Cytisatin        | C32H44O11 | Flavonoids |
| 7.8E-04 | 199.097             | [M-H]+  | 200.105             | 2           | Deoxycoumarin acid | C30H18O7 | Lipids |
| 8.2E-04 | 283.010             | [M+Na-2H]+| 242.236             | 2           | Glucose(2:6,10b)-(11,12-dihydroxy-2,6-farnesidosesoyl | C23H36O10 | Lipids |
| 8.4E-04 | 869.480             | [M+Cl]  | 833.521             | 20          | PS (18:1(9z):22:6(4z,7z,10z,13z,16z,19z)) | C46H76O30P | Lipids |
| 6.0E-04 | 603.289             | [M+Cl]  | 567.317             | 8           | PS (10:0:10:0) | C26H50O10P | Lipids |
| 4.2E-04 | 449.190             | [M+K-2H]+| 411.239             | 10          | PC (O-8:0:2:0) | C18H38N7P | Lipids |
| 8.3E-04 | 431.196             | [M+Cl]  | 397.223             | 7           | PE (12:0:12:0) | C17H36N7P | Lipids |
| 1.4E-04 | 251.003             | [M+H]+  | 250.000             | 18          | Glycerate        | C6H10O8 | Photoreception |
| 3.9E-04 | 251.013             | [M+H]+  | 251.106             | 3           | Hydroxyrosyl 1-O-glucose | C6H11O5 | Flavonoids |
| 2.7E-04 | 249.112             | [M+H-2H]+| 248.105             | 0           | Phenyl caffeate | C14H10O4 | Flavonoids |
| 1.2E-04 | 350.988             | [M+K]+  | 312.022             | 7           | Gamma-Glutamy-Se-methylselenocysteine | C9H16N2O2Se | Redox |
| 4.4E-04 | 267.087             | [M-H]+  | 268.088             | 21          | Cysteinyl-Phenylalanine | C12H16N2OSS | Redox |
| 6.9E-04 | 665.080             | [M-H]+  | 664.093             | 30          | Deaminonitroacetone | C21H26N1O1P2 | Redox |
| 1.1E-04 | 493.077             | [M+Na]+ | 470.088             | 0           | Phenyltyrosine | C23H19N2O7 | Amino acids |
| 6.6E-04 | 423.159             | [M+Na-2H]+| 402.189             | 10          | D-Linolenic acid 5-(6'-malonylglucoside) | C19H30O9 | Terpenoids |
| 5.1E-04 | 467.166             | [M-H]+  | 468.178             | 11          | Dakunol D | C26H28O8 | Terpenoids |
| 4.2E-04 | 433.192             | [M-H]+  | 432.197             | 5           | K-Faranopeptatin | C24H32OSS | Terpenoids |
| 1.1E-04 | 331.001             | [M-H]+  | 331.001             | 4           | Unknown |
| 7.3E-04 | 330.987             | [M-H]+  | 330.987             | 4           | Unknown |
| 8.1E-04 | 330.957             | [M-H]+  | 329.987             | 4           | Unknown |
| 3.0E-05 | 249.986             | [M-H]+  | 249.986             | 4           | Unknown |
| 1.7E-04 | 997.443             | [M-H]+  | 997.443             | 2           | Unknown |
| 7.4E-04 | 1001.458             | [M-H]+  | 1001.458             | 2           | Unknown |
| 8.4E-04 | 1100.454             | [M-H]+  | 1100.454             | 3           | Unknown |
| 7.2E-04 | 1100.454             | [M-H]+  | 1100.454             | 3           | Unknown |
| 7.2E-04 | 1100.454             | [M-H]+  | 1100.454             | 3           | Unknown |

Table S2. Putative identification of metabolic markers detected by UPLC-Q-TOF.
| Peak  | m/z (ppm) | Intensity | Retention Time (min) | Formula | Name | Database |
|-------|-----------|-----------|----------------------|---------|------|----------|
| 1.0E-05 | 189.074  | 1.8       | 72 hours + Hpa       | [M-H2O-H]- | 208.089 | 17 Chalcone | C15H12O |
| 1.1E-03 | 417.107  | 2.3       |                      | [M-H]-  | 418.126 | 29 4'-Hydroxy-3,5,6,7,3',5'-hexamethoxyflavone | C21H22O9 |
| 8.0E-03 | 557.298  | 2.9       |                      | [M-H]-  | 558.298 | 12 Denticulaflavonol | C35H42O6 |
| 1.7E-03 | 454.203  | 3.0       |                      | [M-H]-  | 455.212 | 2 5-Ribosylparomamine | C17H33N3O11 |
| 6.4E-04 | 402.089  | 1.7       |                      | [M-H]-  | 403.097 | 1 3-Methylpentyl glucosinolate | C13H25NO9S2 |
| 5.4E-03 | 488.069  | 1.7       |                      | [M+2Na-H]++ | 403.097 | 0 3-Methylpentyl glucosinolate | C13H25NO9S2 |
| 2.7E-04 | 249.112  | 3.4       |                      | [M+Na-H]- | 228.136 | 5 (-)-11-hydroxy-9,10-dihydrojasmonic acid | C12H20O4 |
| 1.1E-03 | 119.074  | 0.8       |                      | [M+H-H2O]+ | 136.0749 | 15 Tetrahydropteridine | C6H8N4 |
| 3.6E-03 | 221.983  | 0.4       |                      | [M-H2O-H]- | 241.008 | 29 3-Mercaptolactate-cysteine disulfide | C6H11NO5S2 |
| 2.6E-04 | 1044.112 | 5.4       |                      | [M-H]-  | 1043.097 | 1 3-Methylpentyl glucosinolate | C13H25NO9S2 |

* Adducts: type of ion generated by electrospray ionization; Appm: difference between observed and theoretical monoisotopic masses; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PG: Phosphatidylserine.

** Conditions for which the metabolic markers showed a statistically significant accumulation between saCO2 and aCO2

** a P values indicate levels of significance from FDR adjusted ANOVA and subsequent two-factor ANOVA (P < 0.01)

** c accurate m/z values with their corresponding retention time (RT) detected by UPLC-qTOF-MS

** d predicted parameters from the METLIN database using the detected accurate m/z

** e putative metabolites and their corresponding pathways were validated by information from the PubMed chemical database

** * putative metabolites that are oxidised

** ** Adducts: type of ion generated by electrospray ionization; Appm: difference between observed and theoretical monoisotopic masses; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PG: Phosphatidylserine.
Methods S1: supplemental Materials and Methods

Chemicals and reagents. All chemicals and solvents (higher analytical MS grade) used in this study were purchased from Sigma-Aldrich (UK), except JA which was obtained from OlChemim (http://www.olchemim.cz/).

Targeted quantification of hormones. SA and JA were quantified by UPLC-Q-TOF-MS\(^\text{E}\), using a previous method (Pétriacq et al., 2016). Briefly, phytohormones were double-extracted from frozen leaf material (10 mg dry weight) in a total volume of 1.5 mL of ethyl acetate. Each biologically replicated sample consisted of 4 pooled leaves of similar size and age from different plants. Hormones were quantified by UPLC-Q-TOF-MS\(^\text{E}\) in negative electrospray ionization mode (ESI\(^-\)), using standard curves of pure SA and JA. Compound identity was verified by the following fragmentation patterns: SA, 137→93; and JA, 209→59.

Untargeted metabolic profiling by UPLC-qTOF-MS\(^\text{E}\). Plant metabolic profiles at saCO\(_2\) or aCO\(_2\) were analysed after application of DC. Plants in the 8-leaf stage were inoculated with \textit{Hpa} or water (mock), after which leaf tissue of 4 plants from each pot were pooled as one biological replicate. Replicates (n = 3) were collected in the middle of the photoperiod and snap frozen in liquid nitrogen at 24 hpi and 72 hpi. UPLC-Q-TOF-MS\(^\text{E}\) analysis of methanol extracts was carried out as described previously (Pétriacq et al., 2016), using the following modifications: high-resolution full-scan mass spectrometry was performed with a SYNAPT G2 HDMS Q-TOF mass spectrometer (Waters), coupled to a UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm, Waters) with a guard column (VanGuard, 2.1 x 5 mm, 1.7 µm, Waters) for separation of compounds at a flow rate of 400 µL min\(^{-1}\). The mobile phase consisted of A; water with 0.05% formic acid, and B; acetonitrile with 0.05% formic acid with a gradient applied: 0 – 3 min 5 – 35 % B, 3 – 6 min 35 – 100 % B, holding at 100 % B for 2 min, 8 – 10 min, 100 – 5 % B. The column temperature was kept at 45 °C with an injection volume of 10 µL. Buffer (50% methanol) was injected between treatments and between ESI\(^-\) and ESI\(^+\) ionization modes for stabilization of the electrospray ionization source.

Ions were detected over a mass range of 50 – 1200 Da, using a scan time of 0.2 s (ESI\(^-\) and ESI\(^+\)) with the instrument operating in sensitivity mode for the MS full scan (\textit{i.e.} without collision energy). Collision energy was ramped in the transfer cell from 5 to 45 eV (MS\(^\text{E}\)), using the following conditions:

|                  | ESI\(^-\) | ESI\(^+\) |
|------------------|----------|----------|
| Capillary voltage (kV) | - 3      | + 3      |
| Sampling cone voltage (V) | - 25     | + 25     |
| Extraction cone voltage (V) | - 4.5    | + 10     |
Prior to analysis, the Q-TOF detector was calibrated with a solution of sodium formate. During each run, accurate mass measurements were ensured by infusing leucine enkephalin peptide as an internal reference (10 s scan frequency, cone voltage of 40 V and a capillary voltage of 3 kV). The system was controlled by MassLynx v 4.1 software (Waters).

**Statistical analysis of metabolic profiling data.** Raw files obtained from MassLynx were converted into CDF format, using the Databridge function in MassLynx (v. 4.1). Subsequent alignment and integration of metabolic peaks were performed in R (v 3.1.3), using XCMS (Smith et al., 2006). Peaks were retained for analysis when present in all bioreplicates ($k = 3$), at a threshold intensity of 10 ($I = 10$) and at maximum resolution range of 20 ppm. Peak values from each run were normalised for total ion current (TIC). For each sample, normalised peak values were corrected for dry weight, generating a dataset of 5683 $m/z$ values in ESI$^+$ and 4479 $m/z$ values in ESI$^-$.

Global differences in metabolic signals between treatment/point combinations was visualised for anions (ESI$^-$) and cations (ESI$^+$) separately by principal component analysis (PCA, Fig. S5), using MetaboAnalyst online (v. 3.0; http://www.metaboanalyst.ca; Xia et al., 2015) on median-normalised, cube-root-transformed and Pareto-scaled data. To select ions that are induced by $saCO_2$ directly or primed by $saCO_2$ for augmented induction following $Hpa$ inoculation, ESI$^-$ and ESI$^+$ datasets were analysed separately for statistically significant differences between all CO$_2$/treatment/time-point combinations by one-way ANOVA ($P < 0.01 +$ Benjamini-Hochberg false discovery rate correction, FDR; see Fig. S6), using MarVis (v. 1.0; http://marvis.gobics.de; Kaever et al., 2012). From each ionization mode, 133 statistically significant markers were combined into one dataset of 266 markers for successive 2-way ANOVA ($P < 0.01$), using MeV (v. 4.9.0; http://mev.tm4.org). Heatmaps project TIC- normalised ion current (NIC) values, relative to the average and standard deviation of all NIC values across all samples: Value =  $(NIC - \text{mean}) / \text{SD}$. For each time-point (24 and 72 hpi), this analysis resulted in 3 subsets of markers, whose intensity was influenced by CO$_2$, $Hpa$, or the CO$_2$ x $Hpa$ interaction (Fig. S6b). Hierarchal clustering (Pearson's correlation; MeV) allowed visual selection of ion clusters that are induced directly by $saCO_2$ or primed for augmented induction after $Hpa$ inoculation, as detailed in Fig. S6c. Putative identities of the selected ion markers were based on $m/z$ values at stringent accuracy (< 30 ppm), the using METLIN chemical database (Smith et al., 2005).

| Source Temperature ($°C$) | 120 | 120 |
|---------------------------|-----|-----|
| Desolvation Temperature ($°C$) | 350 | 350 |
| Desolvation gas flow (L h$^{-1}$) | 800 | 800 |
| Cone gas flow (L h$^{-1}$) | 60 | 60 |
References

Kaever A, Landesfeind M, Possienke M, Feussner K, Feussner I, Meinicke P. 2012. MarVis-Filter: ranking, filtering, adduct and isotope correction of mass spectrometry data. Journal of biomedicine & biotechnology 2012: 263910.

Pétriacq P, Ton J, Patrit O, Tcherkez G, Gakière B. 2016. NAD acts as an integral regulator of multiple defense layers. Plant Physiology 172: 1465–1479.

Smith CA, O’Maille G, Want EJ, Qin C, Trauger SA, Brandon TR, Custodio DE, Abagyan R, Siuzdak G. 2005. METLIN: a metabolite mass spectral database. Therapeutic Drug Monitoring 27: 747–751.

Smith CA, Want EJ, O’Maille G, Abagyan R, Siuzdak G. 2006. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Analytical Chemistry 78: 779–787.

Xia J, Sinelnikov I V., Han B, Wishart DS. 2015. MetaboAnalyst 3.0—making metabolomics more meaningful. Nucleic Acids Research 43: W251–W257.