

# **Negative regulation of plastidial isoprenoid pathway by herbivore-induced $\beta$ -cyclocitral in *Arabidopsis thaliana***

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## **MATERIALS AND METHODS**

### **Extraction of plant metabolites**

For  $\beta$ -carotene analysis, ~100 mg pulverized fresh plant material was extracted with 1.5 mL acetone for 6 h in the dark at 4<sup>0</sup> C with continuous shaking. Following a short centrifugation at 4<sup>0</sup> C (3500 g, 5 min in an Eppendorf micro centrifuge), the extract was mixed with water in a ratio of 4:1 (v/v) prior to HPLC analysis. For the analysis of  $\beta$ -carotene oxidation products, 100 mg fresh pulverized leaf tissue was extracted with 1 mL methanol. The supernatant was obtained by centrifugation at 4<sup>0</sup> C (15000 g, 20 min) as above and analyzed by LC-MS/MS.

### **LC-MS/MS analysis of MEP pathway intermediates**

For the separation of MEP pathway intermediates an X-Bridge<sup>Tm</sup> amide column (2.5  $\mu$ m, 150 mm x 2.1 mm) was used. The mobile phases used were 20 mM ammonium acetate, pH 10.5 (solvent A) and 80% (v/v) acetonitrile containing 20 mM ammonium acetate, pH 10.5 (solvent B). Separation was achieved at a flow rate of 0.5 ml min<sup>-1</sup> and a column temperature of 25<sup>0</sup> C. The solvent gradient profile followed an initial isocratic separation with 100% B (5 min) followed by a reduction to 84% B by 11 min and a wash step to 60% B by 20 min. Finally, the column was equilibrated under initial conditions (100% B) for 15 min prior to the next analysis. The mass spectrometer was used in negative ionization mode using instrument settings, data acquisition, and processing as previously described (1). Identification of MEP pathway intermediates was confirmed by the injection of standards from Sigma-Aldrich (Taufkirchen, Germany ([<http://www.sigmaaldrich.com/>])).

### **HPLC analysis of carotenoids and oxidation products**

For  $\beta$ -carotene analysis, solvent A was 1 mM NaHCO<sub>3</sub> in water and solvent B was 100% acetone. The gradient consisted of 65% B isocratically for 4 min, followed by a rise to 90% B by 12 min. Solvent B was increased to 100% by 20 min and held constant for 2 min. The column was then restored to initial conditions by reducing B to 65% by 22.5 min and holding constant until 25 min. Flow rate was held constant at 1.5 ml·min<sup>-1</sup>.  $\beta$ -carotene elution was monitored at 450 nm (retention time ~15.1 minutes). Its identity was confirmed by the injection of a  $\beta$ -carotene standard from Sigma-Aldrich.

The mobile phases for were 0.5% (v/v) formic acid (solvent A) and 100% acetonitrile (solvent B). Separation was achieved at a flow rate of 1.1 ml·min<sup>-1</sup>. The solvent gradient profile consisted of an isocratic phase at 95% A for 2 min followed by a gradient to 50% A by 4 min and finally reaching 100% B by 8.1 min. The column was then equilibrated under the initial conditions (95% B) for 2.9 min. Identification was confirmed by the injection of βCC and βI standards from Sigma-Aldrich and Alfa Aesar (Ward Hill, MA, USA) (<http://www.alpha.com/>), respectively.

### **DXS enzyme assay**

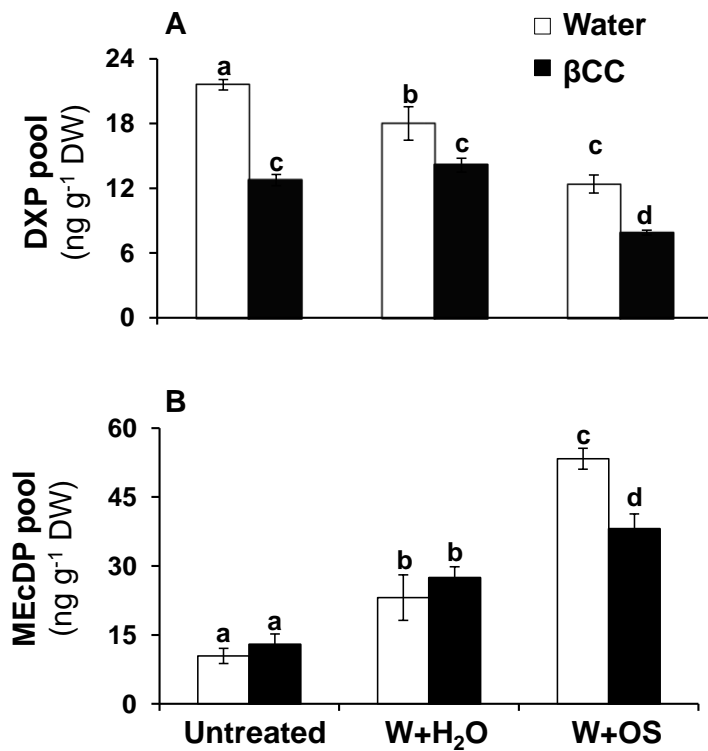
Protein was extracted in 1 mL of extraction buffer at 4°C for 15 min by gently mixing the suspension on a vertical rotator (Stuart rotator SB3; VWR International) at 20 rpm. The suspension was then centrifuged at 16,000g for 20 min at 4°C. For the *in vitro* inhibition assay, enzyme was extracted from untreated *A. thaliana* plants and the enzyme extract was pre-incubated with βCC for 15 min. To start the enzyme reaction, 30 μl of enzyme extract with or without βCC was added to a final volume of 100 μl of assay buffer and incubated in a dry bath at 25°C for 2 h. The enzyme reaction was stopped by adding 100 μl of chloroform and vortexing vigorously for 5 s. The aqueous phase of this mixture was collected after centrifugation and diluted with 10 ng mL<sup>-1</sup> [3, 4, 5- <sup>13</sup>C<sub>3</sub>] DXP (1:10) as an internal standard. Finally, the enzymatic end product DXP was quantified relative to the internal standard using an Agilent 1200 HPLC system (Agilent Technologies) connected to an API 3200 triple quadrupole mass spectrometer (AB Sciex) following the method described under LC-MS/MS analysis of MEP pathway intermediates. The DXP produced by the DXS enzyme reaction was quantified using external standard curves and was normalized to the [<sup>13</sup>C<sub>3</sub>]- DXP internal standard.

### **Determination of reactive oxygen species (ROS) by fluorescence**

100 mg flash frozen, pulverized leaf tissue was extracted with 1 mL of 10 mM Tris-HCl (pH 7.2) and centrifuged at 12,000x g for 20 min at 4°C. The supernatant was diluted (1:40) with 10 mM Tris-HCl (pH 7.2). A 1 mM DCFDA stock solution was added to the diluted supernatant at a final concentration of 10 μM. This assay mix was incubated in complete darkness at room temperature for 10 min. Fluorescence was measured in a 96 well plate at 485 nm excitation and 530 nm emission, and at optimal gain in a TECAN infinite® 200 pro plate reader (Tecan, Zürich).

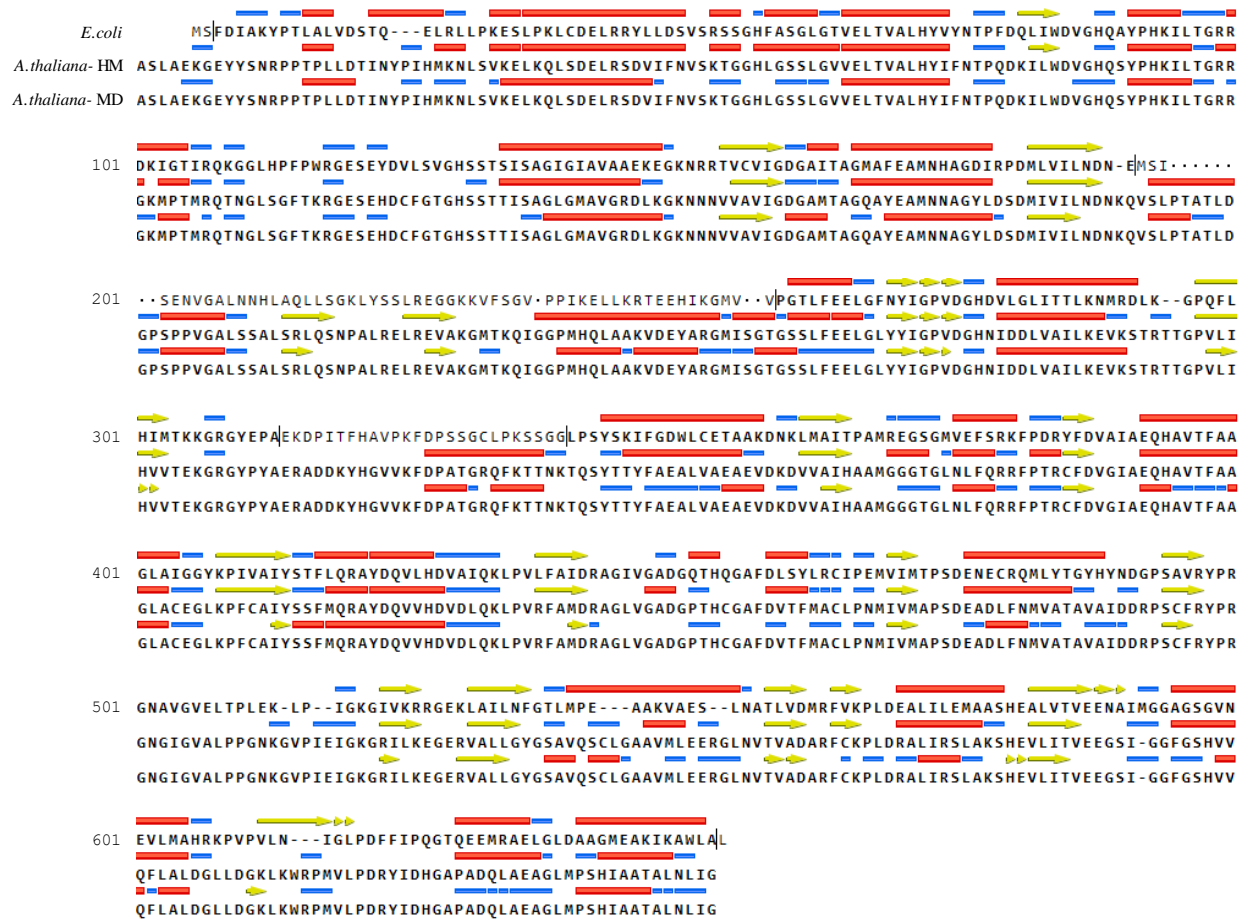
## **Computational Modeling of *A. thaliana* DXS**

MD simulations were conducted to assess the dynamic behavior of the DXS-TPP complex with or without ligand. Semi-empirical (AM1) with bond charge correction (BCC) charges for pyruvate and  $\beta$ CC were calculated using Chimera developed by University of California, San Francisco (2). Protein-ligand complexes were prepared using tleap and MD simulations were conducted in AMBER18 (3). Models were initially solvated with TIP3P water solvent and counter ions were added in a system with periodic boundary conditions. After energy minimization, the system was heated at 300° K within 1 ns followed by a 2 ns long stage under NPT (number of particles, pressure, and temperature) ensemble (P = 1 bar) allowing density balance. The final production stage was conducted at NVT (number of particles, volume, and temperature) during 100 ns. The particle mesh Ewald method was used for describing electrostatic interactions within a 8 Å short-range cutoff. The SHAKE algorithm was used to fix all hydrogen atom positions. The time step was fixed to 2 fs. Trajectory analysis, including RMSD calculations and cluster analysis, was conducted using CPPTRAJ (4).

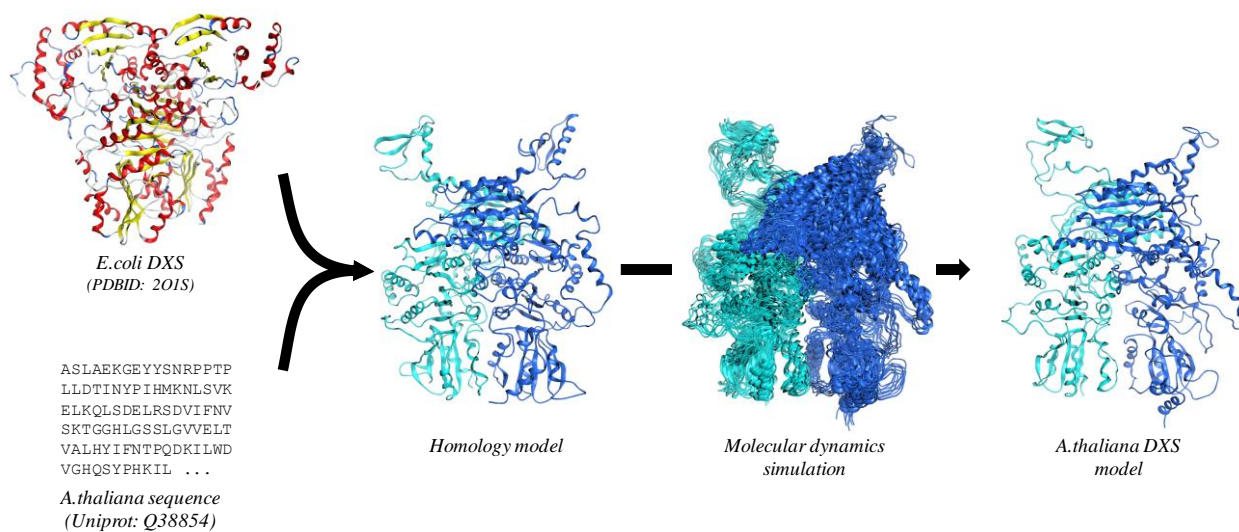


**Figure S1: βCC treatment coupled with mechanical wounding and simulated herbivory treatment reduces accumulation of DXP and of MEcDP only after βCC plus simulated herbivory.**

Rosette stage *A. thaliana* plants treated with βCC or water were further wounded (W) with a fabric pattern wheel and treated with either sterile water (H<sub>2</sub>O) or oral secretion (OS) from *Spodoptera littoralis* larvae. Tissues were harvested 4 h after the treatment and analyzed for DXP and MEcDP accumulation. **(A)** When βCC treatment was coupled with mechanical wounding (W+H<sub>2</sub>O) and simulated herbivory (W+OS), DXP level was decreased more in βCC +W+OS treated plants than in βCC+ untreated plants or βCC+W+H<sub>2</sub>O plants. **(B)** MEcDP level was not significantly changed when βCC treatment was applied to untreated plants or coupled with W+H<sub>2</sub>O treatment. However, βCC +W+OS treated plants accumulated significantly less MEcDP as compared to water + W+OS treated plants. Values are means (± SE) of 4 replicate plants. Different letters indicate a significant difference at  $P \leq 0.05$  by one-way ANOVA.

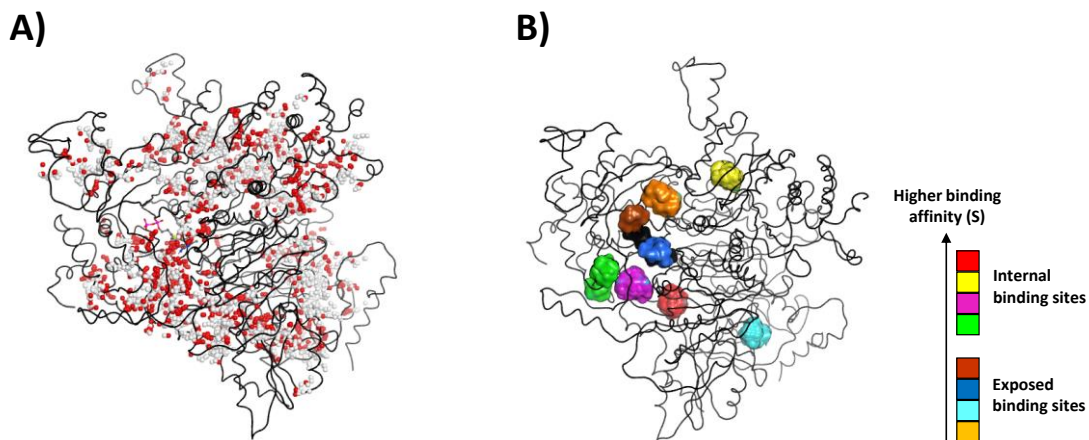


**Figure S2: Alignment of amino acid sequences from *E. coli* DXS and *A. thaliana* DXS and secondary structure comparison.** The secondary structure predicted by MOE software (Chemical Computing Group) is shown above the amino acid sequences indicating the regions with  $\alpha$ -helix (red), turn (blue) and  $\beta$ -sheet (yellow). The secondary structure of the *A. thaliana* model obtained by homology modelling (*A. thaliana*-HM) is mostly preserved after the refinement by molecular dynamics simulations (*A. thaliana*-MD), evincing the stability of the structural model.



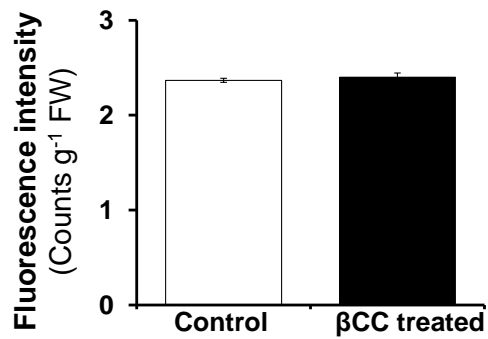
**Figure S3: Schematic representation of the procedure followed to obtain the structural model of the *A. thaliana* DXS protein.**

The three-dimensional structure of *A. thaliana* DXS protein was predicted by homology modeling since no crystallographic data is available in the Protein Data Bank. The amino acid sequence of *AtDXS* was retrieved from UniProt (ID:Q38854). MOE software (Chemical Computing Group) was used for the acquisition of 10 structural models considering the *E. coli* DXS structure obtained from X-ray crystallography as template (PDBID: 201S). The final model was therefore refined by molecular dynamic simulation during 100 ns using AMBER18. The most representative conformer was identified by applying cluster analysis on the last 30 ns of the simulation.



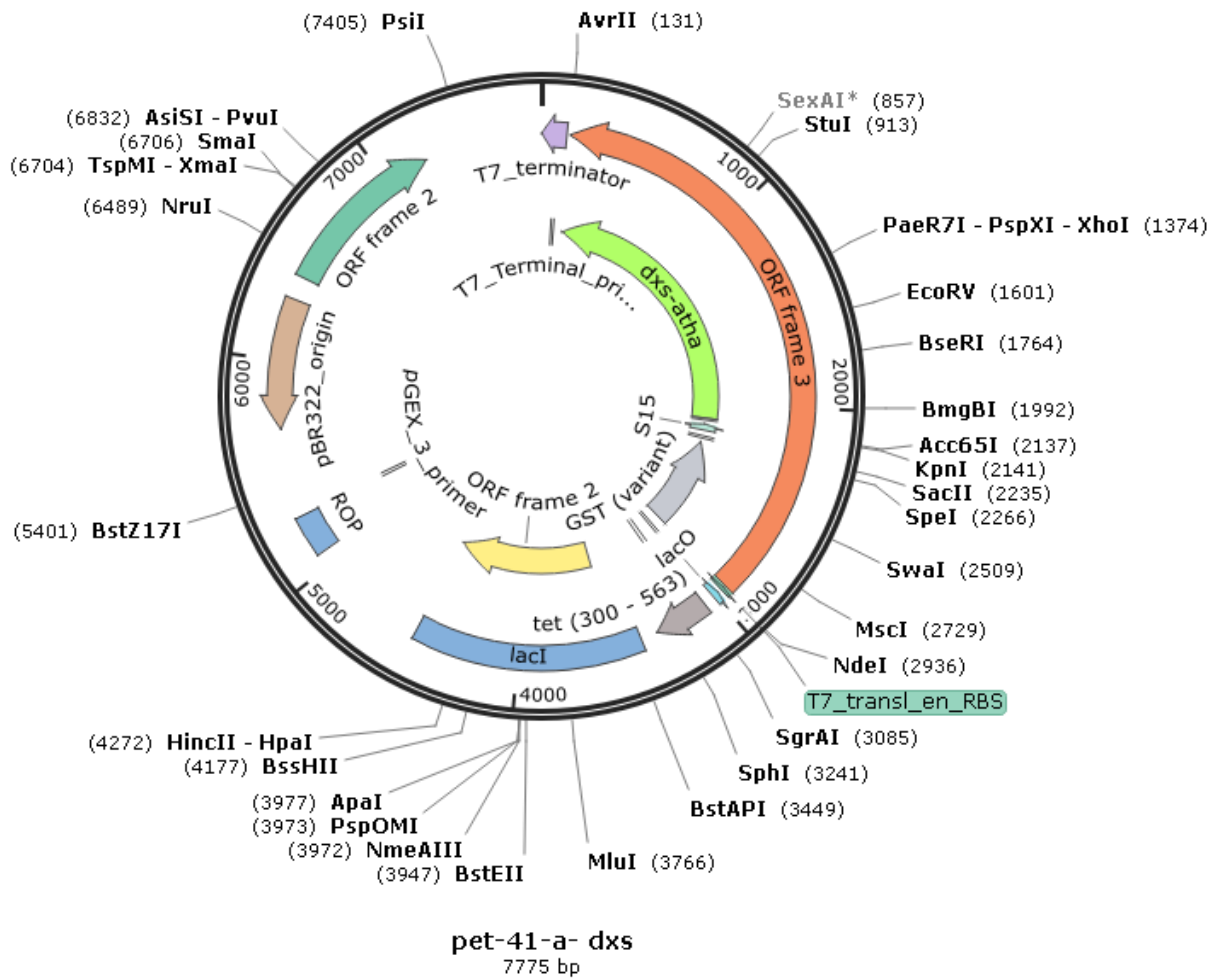
**Figure S4. Determination of possible binding sites of  $\beta$ CC by blind docking.** (A) Graphical representation of alpha spheres generated during the study of possible active sites, colored by hydrophobicity (hydrophobic sites are red colored and hydrophilic in white). This process was performed using the Site Finder tool in MOE software. B) Color surfaces corresponding to the best ranked  $\beta$ CC binding sites predicted by blind docking.





**Figure S5: βCC treatment did not induce ROS accumulation in *A. thaliana* plants.**

Accumulation of reactive oxygen species remains unaltered after βCC treatment. The treatment consisted of placing 500 μL of βCC in a 3 L desiccator with the plants for 4 h. Values are means ( $\pm$  SE) of three replicate plants. Significant difference was determined by Student's t test (Student's t test;  $t_{(4)} = 0.024$ ;  $p = 0.982$ ) at  $p \leq 0.05$ .



**Figure S6: Map of expression vector showing the insertion site of *A. thaliana* DXS.**

## Tables

**Table S1:** Primers used for qPCR analysis of genes whose expression was analyzed after mechanical wounding and simulated herbivory treatments.

Primer Name	Primer Sequence
<i>AtDXS</i>	Forward: 5' TCGCAAAGGGTATGACAAAG 3' Reverse: 5' CAGTCCCGCTTATCATTCC 3'
<i>AtMDS</i>	Forward:5' CATCGTTTAGAGCCAGGGTATCC 3' Reverse:5' TGAAGTAACACATCGCCATCGG 3'
<i>AtHDS</i>	Forward:5' CAGAATGCGTAACACTAAGAC3 ' Reverse:5' GAGAACCACCTACATATCCG3 '
<i>AtHDR</i>	Forward:5' TCGTGCGGGAGAATCATC 3' Reverse:5' TCTTACGGAACACCTTGGC 3'
<i>AtBAP1</i>	Forward:5' CGAATCGAGAAGAAGCAATCC 3' Reverse:5' ACCTTCAGGTGAATACCTTCC 3'
<i>AtTCH4</i>	Forward:5' GAAACTCCGCAGGAACAGTC 3' Reverse:5' TGTCTCCTTTGCCTTGTGTG 3'
<i>AtHP1</i>	Forward:5' GACACGACGCCTACAGACAA 3' Reverse:5' CAACATCTCCATCGCATCAG 3'
<i>AtAPT1</i>	Forward:5' GTTGCAGGTGTTGAAGCTAGAGGT 3' Reverse:5' TGGCACCAATAGCCAACGCAATAG 3'

**Table S2: Analysis of photosynthetic rate after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 2.619$ ;  $p = 0.026$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	0.8275
Untreated 0 min× Untreated 60 min	0.7694
Untreated 0 min× Untreated 90 min	0.2844
Untreated 0 min× Untreated 180 min	0.09967
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	1
Untreated 30 min× Untreated 90 min	0.9941
Untreated 30 min× Untreated 180 min	0.8905
Untreated 30 min× W+H <sub>2</sub> O 30 min	<b>0.006728</b>
Untreated 30 min× W+OS 30 min	<b>0.01148</b>
Untreated 60 min× Untreated 90 min	0.9977
Untreated 60 min× Untreated 180 min	0.9278
Untreated 60 min× W+H <sub>2</sub> O 60 min	<b>0.001059</b>
Untreated 60 min× W+OS 60 min	<b>0.03784</b>
Untreated 90 min× Untreated 180 min	0.9999
Untreated 90 min× W+H <sub>2</sub> O 90 min	<b>0.001044</b>
Untreated 90 min× W+OS 90 min	<b>0.02547</b>
Untreated 180 min× W+H <sub>2</sub> O 180 min	<b>0.001238</b>
Untreated 180 min× W+OS 180 min	<b>0.01435</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	<b>0.2598</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	0.0828
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	0.3754
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	0.753
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.9998
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	0.9971
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	0.9978
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.9096
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	0.922
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.9998
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.9619
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	0.9939
W+ OS 0 min × W+ OS 30 min	0.3636
W+ OS 0 min × W+ OS 60 min	0.7306
W+ OS 0 min × W+ OS 90 min	0.9749
W+ OS 0 min × W+ OS 180 min	0.9974
W+ OS 30 min × W+ OS 60 min	0.9998
W+ OS 30 min × W+ OS 90 min	0.9577
W+ OS 30 min × W+ OS 180 min	0.8538
W+ OS 60 min × W+ OS 90 min	0.9996
W+ OS 60 min × W+ OS 180 min	0.9919
W+ OS 90 min × W+ OS 180 min	1

**Table S3: Analysis of DXP pool size after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 5.23$ ;  $p = 0.0003$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	0.6445
Untreated 0 min× Untreated 60 min	0.7697
Untreated 0 min× Untreated 90 min	1
Untreated 0 min× Untreated 180 min	0.2015
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	1
Untreated 30 min× Untreated 90 min	0.6412
Untreated 30 min× Untreated 180 min	0.9979
Untreated 30 min× W+H <sub>2</sub> O 30 min	0.05598
Untreated 30 min× W+OS 30 min	<b>0.000588</b>
Untreated 60 min× Untreated 90 min	0.7668
Untreated 60 min× Untreated 180 min	0.9891
Untreated 60 min× W+H <sub>2</sub> O 60 min	<b>0.01771</b>
Untreated 60 min× W+OS 60 min	<b>0.001497</b>
Untreated 90 min× Untreated 180 min	0.1996
Untreated 90 min× W+H <sub>2</sub> O 90 min	<b>0.0349</b>
Untreated 90 min× W+OS 90 min	0.05108
Untreated 180 min× W+H <sub>2</sub> O 180 min	<b>1.38E-06</b>
Untreated 180 min× W+OS 180 min	<b>3.43E-07</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	<b>0.9103</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	0.5332
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	<b>0.03448</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	<b>0.002388</b>
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.9994
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	0.5112
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	0.08147
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	0.7467
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	0.898
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.3066
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	0.9933
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.9864
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	1
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	0.9999
W+ OS 0 min × W+ OS 30 min	0.08124
W+ OS 0 min × W+ OS 60 min	0.1086
W+ OS 0 min × W+ OS 90 min	0.05048
W+ OS 0 min × W+ OS 180 min	<b>0.000564</b>
W+ OS 30 min × W+ OS 60 min	1
W+ OS 30 min × W+ OS 90 min	1
W+ OS 30 min × W+ OS 180 min	0.6348
W+ OS 60 min × W+ OS 90 min	1
W+ OS 60 min × W+ OS 180 min	0.5475
W+ OS 90 min × W+ OS 180 min	0.7639

**Table S4: Analysis of flux through MEP pathway after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** One-way ANOVA showed a significant difference in DXP pool size between untreated and W+OS treated plants ( $F_{2,27}=6.733$ ;  $p=0.004$ ). Significance of differences was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Treatments</b>	<b><i>p</i>- value</b>
Untreated × W+H <sub>2</sub> O	0.2973
Untreated × W+OS	<b>0.00306</b>
W+H <sub>2</sub> O × W+OS	0.1022



**Table S5: Analysis of DXS relative transcript abundance after mechanical wounding (W+H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 3.32$ ;  $p = 0.007$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	1
Untreated 0 min× Untreated 60 min	0.9998
Untreated 0 min× Untreated 90 min	0.9828
Untreated 0 min× Untreated 180 min	1
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	0.9992
Untreated 30 min× Untreated 90 min	0.9676
Untreated 30 min× Untreated 180 min	1
Untreated 30 min× W+H <sub>2</sub> O 30 min	1
Untreated 30 min× W+OS 30 min	0.5981
Untreated 60 min× Untreated 90 min	1
Untreated 60 min× Untreated 180 min	1
Untreated 60 min× W+H <sub>2</sub> O 60 min	0.7574
Untreated 60 min× W+OS 60 min	0.6189
Untreated 90 min× Untreated 180 min	0.9968
Untreated 90 min× W+H <sub>2</sub> O 90 min	0.5659
Untreated 90 min× W+OS 90 min	<b>0.007805</b>
Untreated 180 min× W+H <sub>2</sub> O 180 min	<b>0.000708</b>
Untreated 180 min× W+OS 180 min	<b>0.001629</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	<b>1</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	0.9745
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	0.9914
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	<b>0.001312</b>
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.9034
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	0.9527
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	<b>0.000592</b>
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	0.3522
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	<b>0.02598</b>
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	1
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	<b>0.01703</b>
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.5381
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	1
W+ OS 0 min × W+ OS 30 min	0.5264
W+ OS 0 min × W+ OS 60 min	0.9251
W+ OS 0 min × W+ OS 90 min	0.1029
W+ OS 0 min × W+ OS 180 min	<b>0.002991</b>
W+ OS 30 min × W+ OS 60 min	0.9989
W+ OS 30 min × W+ OS 90 min	0.9925
W+ OS 30 min × W+ OS 180 min	0.3561
W+ OS 60 min × W+ OS 90 min	0.7829
W+ OS 60 min × W+ OS 180 min	0.08844
W+ OS 90 min × W+ OS 180 min	0.9016

**Table S6: Analysis of DXS activity after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 3.58$ ;  $p = 0.004$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	1
Untreated 0 min× Untreated 60 min	1
Untreated 0 min× Untreated 90 min	0.9067
Untreated 0 min× Untreated 180 min	0.837
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	1
Untreated 30 min× Untreated 90 min	0.9045
Untreated 30 min× Untreated 180 min	0.8341
Untreated 30 min× W+H <sub>2</sub> O 30 min	0.9887
Untreated 30 min× W+OS 30 min	0.9996
Untreated 60 min× Untreated 90 min	0.9878
Untreated 60 min× Untreated 180 min	0.9669
Untreated 60 min× W+H <sub>2</sub> O 60 min	0.6676
Untreated 60 min× W+OS 60 min	<b>0.00937</b>
Untreated 90 min× Untreated 180 min	1
Untreated 90 min× W+H <sub>2</sub> O 90 min	0.2339
Untreated 90 min× W+OS 90 min	<b>0.009212</b>
Untreated 180 min× W+H <sub>2</sub> O 180 min	<b>0.03017</b>
Untreated 180 min× W+OS 180 min	<b>0.003162</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	<b>0.9891</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	0.8822
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	0.9614
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	0.5899
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.3027
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	0.4528
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	0.1136
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.9999
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	0.4823
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.9981
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.9043
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	0.9959
W+ OS 0 min × W+ OS 30 min	0.9996
W+ OS 0 min × W+ OS 60 min	<b>0.02571</b>
W+ OS 0 min × W+ OS 90 min	0.2312
W+ OS 0 min × W+ OS 180 min	0.1472
W+ OS 30 min × W+ OS 60 min	<b>0.0049</b>
W+ OS 30 min × W+ OS 90 min	<b>0.06127</b>
W+ OS 30 min × W+ OS 180 min	<b>0.03516</b>
W+ OS 60 min × W+ OS 90 min	0.9883
W+ OS 60 min × W+ OS 180 min	0.9983
W+ OS 90 min × W+ OS 180 min	1

**Table S7: Analysis of MEcDP pool after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 10.54$ ;  $p = 6.769\text{E-}07$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	0.8752
Untreated 0 min× Untreated 60 min	0.413
Untreated 0 min× Untreated 90 min	1
Untreated 0 min× Untreated 180 min	0.996
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	0.9982
Untreated 30 min× Untreated 90 min	0.9818
Untreated 30 min× Untreated 180 min	0.3631
Untreated 30 min× W+H <sub>2</sub> O 30 min	0.9998
Untreated 30 min× W+OS 30 min	0.9873
Untreated 60 min× Untreated 90 min	0.679
Untreated 60 min× Untreated 180 min	0.0825
Untreated 60 min× W+H <sub>2</sub> O 60 min	1
Untreated 60 min× W+OS 60 min	1
Untreated 90 min× Untreated 180 min	0.9451
Untreated 90 min× W+H <sub>2</sub> O 90 min	<b>0.003086</b>
Untreated 90 min× W+OS 90 min	<b>6.69E-05</b>
Untreated 180 min× W+H <sub>2</sub> O 180 min	<b>1.38E-06</b>
Untreated 180 min× W+OS 180 min	<b>5.08E-08</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	<b>0.9951</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	0.5598
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	<b>0.000986</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	<b>1.46E-05</b>
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.9722
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	<b>0.01085</b>
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	<b>0.000175</b>
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	0.1581
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	<b>0.004131</b>
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	1
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.8662
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.9162
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	0.9495
W+ OS 0 min × W+ OS 30 min	1
W+ OS 0 min × W+ OS 60 min	0.3028
W+ OS 0 min × W+ OS 90 min	<b>2.10E-05</b>
W+ OS 0 min × W+ OS 180 min	<b>4.77E-07</b>
W+ OS 30 min × W+ OS 60 min	0.5859
W+ OS 30 min × W+ OS 90 min	<b>7.82E-05</b>
W+ OS 30 min × W+ OS 180 min	<b>1.68E-06</b>
W+ OS 60 min × W+ OS 90 min	<b>0.01799</b>
W+ OS 60 min × W+ OS 180 min	<b>0.000421</b>
W+ OS 90 min × W+ OS 180 min	0.9121

**Table S8: Analysis of IPP/DMADP pool after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA did not show a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 1.4$ ;  $p = 0.2$ ). Therefore, we proceeded to interpret the main effects and found that there were significant effects of treatment ( $F_{2,30} = 5.94$ ,  $p_{\text{treatment}} = 0.006$ ) but no significant effects of time ( $F_{2,30} = 0.775$ ,  $p_{\text{time}} = 0.55$ ). Significance of treatment and time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	0.9994
Untreated 0 min× Untreated 60 min	0.9441
Untreated 0 min× Untreated 90 min	0.9993
Untreated 0 min× Untreated 180 min	1
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	0.9997
Untreated 30 min× Untreated 90 min	1
Untreated 30 min× Untreated 180 min	0.9998
Untreated 30 min× W+H <sub>2</sub> O 30 min	0.7036
Untreated 30 min× W+OS 30 min	1
Untreated 60 min× Untreated 90 min	0.9998
Untreated 60 min× Untreated 180 min	0.9632
Untreated 60 min× W+H <sub>2</sub> O 60 min	0.3624
Untreated 60 min× W+OS 60 min	1
Untreated 90 min× Untreated 180 min	0.9998
Untreated 90 min× W+H <sub>2</sub> O 90 min	0.9159
Untreated 90 min× W+OS 90 min	0.7815
Untreated 180 min× W+H <sub>2</sub> O 180 min	1
Untreated 180 min× W+OS 180 min	1

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	<b>0.9735</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	0.9821
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	0.9993
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	1
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	0.9466
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	0.4019
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.9613
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	0.3371
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.9971
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.09622
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	1
W+ OS 0 min × W+ OS 30 min	0.9717
W+ OS 0 min × W+ OS 60 min	0.9316
W+ OS 0 min × W+ OS 90 min	0.3527
W+ OS 0 min × W+ OS 180 min	1
W+ OS 30 min × W+ OS 60 min	1
W+ OS 30 min × W+ OS 90 min	0.9582
W+ OS 30 min × W+ OS 180 min	0.8736
W+ OS 60 min × W+ OS 90 min	0.9851
W+ OS 60 min × W+ OS 180 min	0.7843
W+ OS 90 min × W+ OS 180 min	0.1941



**Table S9: Analysis of  $\beta$ carotene levels after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 4.2$ ;  $p = 0.001$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	1
Untreated 0 min× Untreated 60 min	0.9998
Untreated 0 min× Untreated 90 min	0.9795
Untreated 0 min× Untreated 180 min	0.9938
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	0.999
Untreated 30 min× Untreated 90 min	0.9595
Untreated 30 min× Untreated 180 min	0.985
Untreated 30 min× W+H <sub>2</sub> O 30 min	<b>0.000111</b>
Untreated 30 min× W+OS 30 min	<b>0.000299</b>
Untreated 60 min× Untreated 90 min	1
Untreated 60 min× Untreated 180 min	1
Untreated 60 min× W+H <sub>2</sub> O 60 min	<b>0.04231</b>
Untreated 60 min× W+OS 60 min	<b>0.000943</b>
Untreated 90 min× Untreated 180 min	1
Untreated 90 min× W+H <sub>2</sub> O 90 min	<b>0.00065</b>
Untreated 90 min× W+OS 90 min	<b>3.39E-05</b>
Untreated 180 min× W+H <sub>2</sub> O 180 min	<b>0.000142</b>
Untreated 180 min× W+OS 180 min	<b>0.000185</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	0.000157
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	<b>0.009557</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	<b>3.07E-05</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	<b>1.10E-05</b>
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.8704
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	0.9998
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	0.9919
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	0.5277
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.3093
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	0.8908
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	1
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.9835
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	1
W+ OS 0 min × W+ OS 30 min	<b>0.000422</b>
W+ OS 0 min × W+ OS 60 min	<b>0.00018</b>
W+ OS 0 min × W+ OS 90 min	<b>1.69E-06</b>
W+ OS 0 min × W+ OS 180 min	<b>1.43E-05</b>
W+ OS 30 min × W+ OS 60 min	1
W+ OS 30 min × W+ OS 90 min	0.5855
W+ OS 30 min × W+ OS 180 min	0.9599
W+ OS 60 min × W+ OS 90 min	0.7757
W+ OS 60 min × W+ OS 180 min	0.9942
W+ OS 90 min × W+ OS 180 min	0.9981

**Table S10: Analysis of violaxanthin levels after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed no significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 1.6$ ;  $p = 0.14$ ); Therefore, we proceeded to interpret the main effects and found that there were significant effects of treatment ( $F_{(\text{treatment}) 2,30} = 23.99$ ;  $p = 5.978\text{E-}07$ ) and time ( $F_{(\text{time}) 2,30} = 33.15$ ;  $p = 1.293\text{E-}10$ ). Significance of treatment and time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	1
Untreated 0 min× Untreated 60 min	0.9283
Untreated 0 min× Untreated 90 min	<b>0.02438</b>
Untreated 0 min× Untreated 180 min	0.06534
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	0.9784
Untreated 30 min× Untreated 90 min	<b>0.04369</b>
Untreated 30 min× Untreated 180 min	0.1108
Untreated 30 min× W+H <sub>2</sub> O 30 min	0.08446
Untreated 30 min× W+OS 30 min	<b>0.005645</b>
Untreated 60 min× Untreated 90 min	0.3896
Untreated 60 min× Untreated 180 min	0.6519
Untreated 60 min× W+H <sub>2</sub> O 60 min	0.1527
Untreated 60 min× W+OS 60 min	0.06666
Untreated 90 min× Untreated 180 min	1
Untreated 90 min× W+H <sub>2</sub> O 90 min	0.324
Untreated 90 min× W+OS 90 min	0.07586
Untreated 180 min× W+H <sub>2</sub> O 180 min	<b>0.03448</b>
Untreated 180 min× W+OS 180 min	<b>0.03058</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	0.04886
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	<b>0.006189</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	<b>3.30E-05</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	<b>5.20E-06</b>
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.9973
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	0.1952
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	<b>0.04655</b>
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	0.9792
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	0.6519
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.2575
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	1
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.9995
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.9987
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	1
W+ OS 0 min × W+ OS 30 min	<b>0.002958</b>
W+ OS 0 min × W+ OS 60 min	<b>0.002179</b>
W+ OS 0 min × W+ OS 90 min	<b>4.24E-06</b>
W+ OS 0 min × W+ OS 180 min	<b>4.55E-06</b>
W+ OS 30 min × W+ OS 60 min	1
W+ OS 30 min × W+ OS 90 min	0.3584
W+ OS 30 min × W+ OS 180 min	0.3729
W+ OS 60 min × W+ OS 90 min	0.4236
W+ OS 60 min × W+ OS 180 min	0.4393
W+ OS 90 min × W+ OS 180 min	1

**Table S11: Analysis of neoxanthin levels after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 7.46$ ;  $p = 1.939\text{E-}05$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	0.8739
Untreated 0 min× Untreated 60 min	0.8029
Untreated 0 min× Untreated 90 min	1
Untreated 0 min× Untreated 180 min	1
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	1
Untreated 30 min× Untreated 90 min	0.8894
Untreated 30 min× Untreated 180 min	0.8544
Untreated 30 min× W+H <sub>2</sub> O 30 min	8.45E-07
Untreated 30 min× W+OS 30 min	2.22E-06
Untreated 60 min× Untreated 90 min	0.8226
Untreated 60 min× Untreated 180 min	0.7787
Untreated 60 min× W+H <sub>2</sub> O 60 min	<b>8.83E-05</b>
Untreated 60 min× W+OS 60 min	<b>3.81E-07</b>
Untreated 90 min× Untreated 180 min	1
Untreated 90 min× W+H <sub>2</sub> O 90 min	<b>1.68E-06</b>
Untreated 90 min× W+OS 90 min	<b>3.25E-07</b>
Untreated 180 min× W+H <sub>2</sub> O 180 min	<b>2.46E-06</b>
Untreated 180 min× W+OS 180 min	<b>9.82E-06</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	5.04E-05
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	<b>0.008067</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	<b>1.86E-06</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	<b>2.18E-06</b>
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.6851
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	0.9632
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	0.973
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	0.1008
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.1141
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	0.587
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	1
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.9997
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	0.9999
W+ OS 0 min × W+ OS 30 min	<b>0.000138</b>
W+ OS 0 min × W+ OS 60 min	<b>3.30E-05</b>
W+ OS 0 min × W+ OS 90 min	<b>3.60E-07</b>
W+ OS 0 min × W+ OS 180 min	<b>8.69E-06</b>
W+ OS 30 min × W+ OS 60 min	0.9999
W+ OS 30 min × W+ OS 90 min	0.47
W+ OS 30 min × W+ OS 180 min	0.9892
W+ OS 60 min × W+ OS 90 min	0.7909
W+ OS 60 min × W+ OS 180 min	1
W+ OS 90 min × W+ OS 180 min	0.9663

**Table S12: Analysis of chlorophyll *a* levels after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 5.74$ ;  $p = 0.0001$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	0.8841
Untreated 0 min× Untreated 60 min	0.9998
Untreated 0 min× Untreated 90 min	1
Untreated 0 min× Untreated 180 min	0.9998
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	0.9952
Untreated 30 min× Untreated 90 min	0.893
Untreated 30 min× Untreated 180 min	0.5363
Untreated 30 min× W+H <sub>2</sub> O 30 min	<b>2.31E-06</b>
Untreated 30 min× W+OS 30 min	<b>9.60E-05</b>
Untreated 60 min× Untreated 90 min	0.9999
Untreated 60 min× Untreated 180 min	0.9659
Untreated 60 min× W+H <sub>2</sub> O 60 min	<b>9.66E-05</b>
Untreated 60 min× W+OS 60 min	<b>0.000647</b>
Untreated 90 min× Untreated 180 min	0.9997
Untreated 90 min× W+H <sub>2</sub> O 90 min	<b>2.38E-07</b>
Untreated 90 min× W+OS 90 min	<b>1.91E-05</b>
Untreated 180 min× W+H <sub>2</sub> O 180 min	<b>0.000241</b>
Untreated 180 min× W+OS 180 min	<b>0.000156</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	0.000134
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	<b>0.000489</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	<b>2.53E-07</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	<b>4.47E-05</b>
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	0.3943
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	1
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	0.9262
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	0.1778
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.9963
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	0.9994
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.6437
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.8123
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	1
W+ OS 0 min × W+ OS 30 min	<b>0.00552</b>
W+ OS 0 min × W+ OS 60 min	<b>0.003187</b>
W+ OS 0 min × W+ OS 90 min	<b>2.03E-05</b>
W+ OS 0 min × W+ OS 180 min	<b>2.89E-05</b>
W+ OS 30 min × W+ OS 60 min	1
W+ OS 30 min × W+ OS 90 min	0.5663
W+ OS 30 min × W+ OS 180 min	0.6491
W+ OS 60 min × W+ OS 90 min	0.6983
W+ OS 60 min × W+ OS 180 min	0.774
W+ OS 90 min × W+ OS 180 min	1



**Table S13: Analysis of chlorophyll *b* levels after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed no significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 1.2$ ;  $p = 0.29$ ); Therefore, we proceeded to interpret the main effects and found that there were significant effects of treatment ( $F_{(\text{treatment}) 2,30} = 15.7$ ;  $p = 2.158\text{E-}05$ ) and time ( $F_{(\text{time}) 2,30} = 3.42$ ;  $p = 0.02$ ). Significance of treatment and time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	0.9983
Untreated 0 min× Untreated 60 min	1
Untreated 0 min× Untreated 90 min	1
Untreated 0 min× Untreated 180 min	1
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	0.9999
Untreated 30 min× Untreated 90 min	0.9968
Untreated 30 min× Untreated 180 min	0.9991
Untreated 30 min× W+H <sub>2</sub> O 30 min	0.08776
Untreated 30 min× W+OS 30 min	0.2842
Untreated 60 min× Untreated 90 min	1
Untreated 60 min× Untreated 180 min	1
Untreated 60 min× W+H <sub>2</sub> O 60 min	0.3549
Untreated 60 min× W+OS 60 min	0.3114
Untreated 90 min× Untreated 180 min	1
Untreated 90 min× W+H <sub>2</sub> O 90 min	<b>0.02171</b>
Untreated 90 min× W+OS 90 min	0.5883
Untreated 180 min× W+H <sub>2</sub> O 180 min	0.3318
Untreated 180 min× W+OS 180 min	0.3032

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	<b>0.3761</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	0.4793
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	<b>0.01838</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	0.3635
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	0.8983
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	1
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	0.9998
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	0.8229
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	1
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	1
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.9064
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.7678
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	1
W+ OS 0 min × W+ OS 30 min	0.7518
W+ OS 0 min × W+ OS 60 min	0.4286
W+ OS 0 min × W+ OS 90 min	0.5451
W+ OS 0 min × W+ OS 180 min	0.3334
W+ OS 30 min × W+ OS 60 min	0.9999
W+ OS 30 min × W+ OS 90 min	1
W+ OS 30 min × W+ OS 180 min	0.9994
W+ OS 60 min × W+ OS 90 min	1
W+ OS 60 min × W+ OS 180 min	1
W+ OS 90 min × W+ OS 180 min	1

**Table S14: Analysis of reactive oxygen species accumulation after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 16.54$ ;  $p = 4.641\text{E-}09$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	1
Untreated 0 min× Untreated 60 min	1
Untreated 0 min× Untreated 90 min	1
Untreated 0 min× Untreated 180 min	1
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	1
Untreated 30 min× Untreated 90 min	1
Untreated 30 min× Untreated 180 min	1
Untreated 30 min× W+H <sub>2</sub> O 30 min	1
Untreated 30 min× W+OS 30 min	1
Untreated 60 min× Untreated 90 min	1
Untreated 60 min× Untreated 180 min	1
Untreated 60 min× W+H <sub>2</sub> O 60 min	0.9868
Untreated 60 min× W+OS 60 min	<b>4.84E-06</b>
Untreated 90 min× Untreated 180 min	1
Untreated 90 min× W+H <sub>2</sub> O 90 min	<b>7.48E-09</b>
Untreated 90 min× W+OS 90 min	<b>8.11E-09</b>
Untreated 180 min× W+H <sub>2</sub> O 180 min	1
Untreated 180 min× W+OS 180 min	0.7958

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	<b>1</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	0.9819
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	<b>5.74E-09</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	1
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.9476
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	<b>9.28E-09</b>
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	1
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	<b>4.60E-10</b>
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.8685
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	<b>3.11E-07</b>
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	<b>1.65E-08</b>
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	1
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	0.8604
W+ OS 0 min × W+ OS 30 min	1
W+ OS 0 min × W+ OS 60 min	<b>5.55E-06</b>
W+ OS 0 min × W+ OS 90 min	<b>6.22E-09</b>
W+ OS 0 min × W+ OS 180 min	0.6113
W+ OS 30 min × W+ OS 60 min	<b>6.39E-06</b>
W+ OS 30 min × W+ OS 90 min	<b>7.04E-09</b>
W+ OS 30 min × W+ OS 180 min	0.6445
W+ OS 60 min × W+ OS 90 min	0.2317
W+ OS 60 min × W+ OS 180 min	<b>0.001277</b>
W+ OS 90 min × W+ OS 180 min	<b>9.17E-07</b>

**Table S15: Analysis of  $\beta$ -cyclocitral levels after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 2.46$ ;  $p = 0.03$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	0.9885
Untreated 0 min× Untreated 60 min	0.9986
Untreated 0 min× Untreated 90 min	0.9886
Untreated 0 min× Untreated 180 min	0.9984
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	1
Untreated 30 min× Untreated 90 min	1
Untreated 30 min× Untreated 180 min	1
Untreated 30 min× W+H <sub>2</sub> O 30 min	0.9474
Untreated 30 min× W+OS 30 min	<b>0.001122</b>
Untreated 60 min× Untreated 90 min	1
Untreated 60 min× Untreated 180 min	1
Untreated 60 min× W+H <sub>2</sub> O 60 min	0.1439
Untreated 60 min× W+OS 60 min	<b>0.02815</b>
Untreated 90 min× Untreated 180 min	1
Untreated 90 min× W+H <sub>2</sub> O 90 min	0.1221
Untreated 90 min× W+OS 90 min	<b>0.02056</b>
Untreated 180 min× W+H <sub>2</sub> O 180 min	0.9085
Untreated 180 min× W+OS 180 min	<b>0.01107</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	<b>0.4089</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	<b>0.02624</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	<b>0.01135</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	0.4729
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.9274
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	0.7838
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	1
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	<b>0.03193</b>
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.8914
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	0.999
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.7229
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.9984
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	0.2597
W+ OS 0 min × W+ OS 30 min	<b>6.84E-05</b>
W+ OS 0 min × W+ OS 60 min	<b>0.004038</b>
W+ OS 0 min × W+ OS 90 min	<b>0.001445</b>
W+ OS 0 min × W+ OS 180 min	<b>0.001413</b>
W+ OS 30 min × W+ OS 60 min	0.8823
W+ OS 30 min × W+ OS 90 min	0.9792
W+ OS 30 min × W+ OS 180 min	0.9802
W+ OS 60 min × W+ OS 90 min	1
W+ OS 60 min × W+ OS 180 min	1
W+ OS 90 min × W+ OS 180 min	1

**Table S16: Analysis of  $\beta$ -ionone levels after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 4.02$ ;  $p = 0.002$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min $\times$ Untreated 30 min	0.6569
Untreated 0 min $\times$ Untreated 60 min	<b>0.001673</b>
Untreated 0 min $\times$ Untreated 90 min	0.1912
Untreated 0 min $\times$ Untreated 180 min	0.1362
Untreated 0 min $\times$ W+H <sub>2</sub> O 0 min	1
Untreated 0 min $\times$ W+OS 0 min	1
Untreated 30 min $\times$ Untreated 60 min	0.1719
Untreated 30 min $\times$ Untreated 90 min	0.9968
Untreated 30 min $\times$ Untreated 180 min	0.9879
Untreated 30 min $\times$ W+H <sub>2</sub> O 30 min	0.9911
Untreated 30 min $\times$ W+OS 30 min	1
Untreated 60 min $\times$ Untreated 90 min	0.6212
Untreated 60 min $\times$ Untreated 180 min	0.7298
Untreated 60 min $\times$ W+H <sub>2</sub> O 60 min	<b>0.000254</b>
Untreated 60 min $\times$ W+OS 60 min	0.9988
Untreated 90 min $\times$ Untreated 180 min	1
Untreated 90 min $\times$ W+H <sub>2</sub> O 90 min	0.9771
Untreated 90 min $\times$ W+OS 90 min	0.756
Untreated 180 min $\times$ W+H <sub>2</sub> O 180 min	0.4616
Untreated 180 min $\times$ W+OS 180 min	0.9387

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	<b>0.1489</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	0.9994
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	0.8174
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	0.9991
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	<b>0.03256</b>
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	0.9548
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	0.4894
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	0.982
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	0.3984
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.9138
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	<b>0.001949</b>
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.9944
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.9997
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	0.9954
W+ OS 0 min × W+ OS 30 min	0.7225
W+ OS 0 min × W+ OS 60 min	<b>0.01195</b>
W+ OS 0 min × W+ OS 90 min	0.9888
W+ OS 0 min × W+ OS 180 min	0.8285
W+ OS 30 min × W+ OS 60 min	0.4859
W+ OS 30 min × W+ OS 90 min	0.9981
W+ OS 30 min × W+ OS 180 min	1
W+ OS 60 min × W+ OS 90 min	0.1268
W+ OS 60 min × W+ OS 180 min	0.3712
W+ OS 90 min × W+ OS 180 min	0.9998



**Table S17: Analysis of *AtTCH4* relative transcript abundance after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 4.36$ ;  $p = 0.001$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	0.9999
Untreated 0 min× Untreated 60 min	1
Untreated 0 min× Untreated 90 min	1
Untreated 0 min× Untreated 180 min	1
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	0.9985
Untreated 30 min× Untreated 90 min	0.9909
Untreated 30 min× Untreated 180 min	1
Untreated 30 min× W+H <sub>2</sub> O 30 min	<b>0.000701</b>
Untreated 30 min× W+OS 30 min	<b>0.000109</b>
Untreated 60 min× Untreated 90 min	1
Untreated 60 min× Untreated 180 min	1
Untreated 60 min× W+H <sub>2</sub> O 60 min	<b>0.001059</b>
Untreated 60 min× W+OS 60 min	0.3195
Untreated 90 min× Untreated 180 min	0.9997
Untreated 90 min× W+H <sub>2</sub> O 90 min	0.5297
Untreated 90 min× W+OS 90 min	0.9996
Untreated 180 min× W+H <sub>2</sub> O 180 min	0.9998
Untreated 180 min× W+OS 180 min	1

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	0.002868
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	<b>0.000534</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	0.2681
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	1
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.9997
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	0.6289
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	<b>0.008436</b>
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	0.9995
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	0.2678
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	<b>0.001631</b>
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	0.3392
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.495
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	0.8936
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	0.9998
W+ OS 0 min × W+ OS 30 min	<b>0.000456</b>
W+ OS 0 min × W+ OS 60 min	0.2075
W+ OS 0 min × W+ OS 90 min	0.9792
W+ OS 0 min × W+ OS 180 min	1
W+ OS 30 min × W+ OS 60 min	0.3094
W+ OS 30 min × W+ OS 90 min	<b>0.008984</b>
W+ OS 30 min × W+ OS 180 min	<b>0.000272</b>
W+ OS 60 min × W+ OS 90 min	0.8309
W+ OS 60 min × W+ OS 180 min	0.1449
W+ OS 90 min × W+ OS 180 min	0.9454

**Table S18: Analysis of *AtBAP1* relative transcript abundance after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 26.82$ ;  $p = 1.213\text{E-}11$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	0.9998
Untreated 0 min× Untreated 60 min	0.9999
Untreated 0 min× Untreated 90 min	1
Untreated 0 min× Untreated 180 min	1
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	1
Untreated 30 min× Untreated 90 min	0.9885
Untreated 30 min× Untreated 180 min	0.998
Untreated 30 min× W+H <sub>2</sub> O 30 min	<b>6.55E-14</b>
Untreated 30 min× W+OS 30 min	<b>4.20E-14</b>
Untreated 60 min× Untreated 90 min	0.9915
Untreated 60 min× Untreated 180 min	0.9987
Untreated 60 min× W+H <sub>2</sub> O 60 min	<b>1.42E-11</b>
Untreated 60 min× W+OS 60 min	<b>5.64E-12</b>
Untreated 90 min× Untreated 180 min	1
Untreated 90 min× W+H <sub>2</sub> O 90 min	<b>1.04E-08</b>
Untreated 90 min× W+OS 90 min	<b>7.34E-09</b>
Untreated 180 min× W+H <sub>2</sub> O 180 min	<b>0.006662</b>
Untreated 180 min× W+OS 180 min	<b>3.42E-06</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	1.18E-13
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	<b>4.66E-11</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	<b>3.96E-09</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	<b>0.004079</b>
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	<b>0.06376</b>
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	<b>0.000382</b>
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	<b>5.25E-10</b>
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	0.9479
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	0.614
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	<b>1.76E-06</b>
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	1
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	<b>0.00039</b>
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	1
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	0.1844
W+ OS 0 min × W+ OS 30 min	4.79E-14
W+ OS 0 min × W+ OS 60 min	1.79E-11
W+ OS 0 min × W+ OS 90 min	<b>2.82E-09</b>
W+ OS 0 min × W+ OS 180 min	<b>2.08E-06</b>
W+ OS 30 min × W+ OS 60 min	<b>0.008456</b>
W+ OS 30 min × W+ OS 90 min	<b>1.64E-05</b>
W+ OS 30 min × W+ OS 180 min	<b>1.77E-08</b>
W+ OS 60 min × W+ OS 90 min	0.4159
W+ OS 60 min × W+ OS 180 min	<b>0.001002</b>
W+ OS 90 min × W+ OS 180 min	0.2455

**Table S19: Analysis of *AtHP1* relative transcript abundance after mechanical wounding (W+ H<sub>2</sub>O) and simulated herbivory (W+OS) in *A. thaliana* plants.** Two-way ANOVA showed a significant interaction between treatment and time ( $F_{(\text{treatment} \times \text{time}) 2,30} = 5.7$ ;  $p = 0.0001$ ). Since the interaction was significant, we refrained from interpreting the main effects of treatment and time. Significance of treatment  $\times$  time was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Interaction</b>	<b><i>p</i>- value</b>
Untreated 0 min× Untreated 30 min	0.9836
Untreated 0 min× Untreated 60 min	0.9967
Untreated 0 min× Untreated 90 min	1
Untreated 0 min× Untreated 180 min	1
Untreated 0 min× W+H <sub>2</sub> O 0 min	1
Untreated 0 min× W+OS 0 min	1
Untreated 30 min× Untreated 60 min	1
Untreated 30 min× Untreated 90 min	0.9682
Untreated 30 min× Untreated 180 min	0.9078
Untreated 30 min× W+H <sub>2</sub> O 30 min	0.9168
Untreated 30 min× W+OS 30 min	0.9903
Untreated 60 min× Untreated 90 min	0.9917
Untreated 60 min× Untreated 180 min	0.9644
Untreated 60 min× W+H <sub>2</sub> O 60 min	1
Untreated 60 min× W+OS 60 min	0.8498
Untreated 90 min× Untreated 180 min	1
Untreated 90 min× W+H <sub>2</sub> O 90 min	1
Untreated 90 min× W+OS 90 min	1
Untreated 180 min× W+H <sub>2</sub> O 180 min	<b>0.03739</b>
Untreated 180 min× W+OS 180 min	<b>7.48E-06</b>

W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 30 min	<b>1</b>
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 60 min	1
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 90 min	1
W+ H <sub>2</sub> O 0 min × W+ H <sub>2</sub> O 180 min	0.08359
W+ H <sub>2</sub> O 0 min × W+ OS 0 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 60 min	0.9986
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 90 min	1
W+ H <sub>2</sub> O 30 min × W+ H <sub>2</sub> O 180 min	<b>0.03981</b>
W+ H <sub>2</sub> O 30 min × W+ OS 30 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 90 min	1
W+ H <sub>2</sub> O 60 min × W+ H <sub>2</sub> O 180 min	0.2034
W+ H <sub>2</sub> O 60 min × W+ OS 60 min	0.9734
W+ H <sub>2</sub> O 90 min × W+ H <sub>2</sub> O 180 min	0.08547
W+ H <sub>2</sub> O 90 min × W+ OS 90 min	1
W+ H <sub>2</sub> O 180 min × W+ OS 180 min	0.08155
W+ OS 0 min × W+ OS 30 min	1
W+ OS 0 min × W+ OS 60 min	0.9992
W+ OS 0 min × W+ OS 90 min	1
W+ OS 0 min × W+ OS 180 min	<b>1.96E-05</b>
W+ OS 30 min × W+ OS 60 min	0.9981
W+ OS 30 min × W+ OS 90 min	1
W+ OS 30 min × W+ OS 180 min	<b>2.44E-05</b>
W+ OS 60 min × W+ OS 90 min	0.994
W+ OS 60 min × W+ OS 180 min	<b>2.83E-06</b>
W+ OS 90 min × W+ OS 180 min	<b>3.52E-05</b>

**Table S20: Analysis of DXP levels after  $\beta$ -cyclocitral ( $\beta$ CC) treatment in *A. thaliana* plants.** One-way ANOVA showed a significant difference in DXP levels between untreated and  $\beta$ CC treated plants ( $F_{3,12}= 5.548$ ;  $p= 0.012$ ). Significance of differences was determined by Tukey's HSD *post-hoc* test at  $p\leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Treatments</b>	<b><i>p</i>- value</b>
Water $\times$ 25 $\mu$ l $\beta$ CC	0.8144
Water $\times$ 125 $\mu$ l $\beta$ CC	<b>0.02727</b>
Water $\times$ 250 $\mu$ l $\beta$ CC	<b>0.03377</b>
25 $\mu$ l $\beta$ CC $\times$ 125 $\mu$ l $\beta$ CC	0.1493
25 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	0.1606
125 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	0.9993

**Table S21: Analysis of MEcDP levels after  $\beta$ -cyclocitral treatment in *A. thaliana* plants.**

One-way ANOVA showed a significant difference in MEcDP levels between untreated and  $\beta$ CC treated plants ( $F_{3,12} = 9.312$ ;  $p = 0.00185$ ). Significance of differences was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Fisher's LSD <i>post hoc</i></b>	
<b>Treatments</b>	<b><i>p</i>- value</b>
Water $\times$ 25 $\mu$ l $\beta$ CC	0.6345
Water $\times$ 125 $\mu$ l $\beta$ CC	0.06257
Water $\times$ 250 $\mu$ l $\beta$ CC	<b>0.0015</b>
25 $\mu$ l $\beta$ CC $\times$ 125 $\mu$ l $\beta$ CC	0.3985
25 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	<b>0.01219</b>
125 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	0.1942



**Table S22: Analysis of IPP/DMADP levels after  $\beta$ -cyclocitral treatment in *A. thaliana* plants.** Welch's ANOVA showed a significant difference in IPP/DMADP levels between untreated and  $\beta$ CC treated plants ( $F_{3,6.362} = 38.74$ ;  $P < 0.00017$ ). Significance of differences was determined by Games Howell *post-hoc* test at  $p \leq 0.05$ .

<b>Games Howell <i>post hoc</i></b>	
<b>Treatments</b>	<b><i>p</i>- value</b>
Water $\times$ 25 $\mu$ l $\beta$ CC	<b>0.0021</b>
Water $\times$ 125 $\mu$ l $\beta$ CC	<b>&lt; 0.0001</b>
Water $\times$ 250 $\mu$ l $\beta$ CC	<b>&lt; 0.0001</b>
25 $\mu$ l $\beta$ CC $\times$ 125 $\mu$ l $\beta$ CC	0.0007
25 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	<b>0.0007</b>
125 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	0.9452

**Table S23: Analysis of *AtDXS* relative transcript abundance after  $\beta$ -cyclocitral treatment in *A. thaliana* plants.** One-way ANOVA showed a significant difference in *AtDXS* relative transcript abundance between untreated and  $\beta$ CC treated plants ( $F_{3,8} = 17.32$ ;  $p = 0.0007$ ). Significance of differences was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Treatments</b>	<b><i>p</i>- value</b>
Water $\times$ 25 $\mu$ l $\beta$ CC	0.6388
Water $\times$ 125 $\mu$ l $\beta$ CC	<b>0.01361</b>
Water $\times$ 250 $\mu$ l $\beta$ CC	<b>0.00082</b>
25 $\mu$ l $\beta$ CC $\times$ 125 $\mu$ l $\beta$ CC	0.07211
25 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	<b>0.00315</b>
125 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	0.1595

**Table S24: Analysis of DXS activity after  $\beta$ -cyclocitral treatment in *A. thaliana* plants.** One-way ANOVA showed a significant difference in DXS activity between untreated and  $\beta$ CC treated plants ( $F_{3,12} = 29.02$ ;  $p < 0.0001$ ). Significance of differences was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Treatments</b>	<b><i>p</i>- value</b>
Water $\times$ 25 $\mu$ l $\beta$ CC	<b>0.0047</b>
Water $\times$ 125 $\mu$ l $\beta$ CC	<b>0.0003</b>
Water $\times$ 250 $\mu$ l $\beta$ CC	<b>&lt; 0.0001</b>
25 $\mu$ l $\beta$ CC $\times$ 125 $\mu$ l $\beta$ CC	0.3628
25 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	<b>0.0020</b>
125 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	<b>0.0394</b>

**Table S25: Analysis of *AtMDS* relative transcript abundance after  $\beta$ -cyclocitral treatment in *A. thaliana* plants.** One-way ANOVA showed a significant difference in *AtMDS* relative transcript abundance between untreated and  $\beta$ CC treated plants ( $F_{3,12}= 36.24$ ;  $P < 0.0001$ ). Significance of differences was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Treatments</b>	<b><i>p</i>- value</b>
Water $\times$ 25 $\mu$ l $\beta$ CC	0.3291
Water $\times$ 125 $\mu$ l $\beta$ CC	0.5714
Water $\times$ 250 $\mu$ l $\beta$ CC	<b>&lt; 0.0001</b>
25 $\mu$ l $\beta$ CC $\times$ 125 $\mu$ l $\beta$ CC	0.9655
25 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	<b>&lt; 0.0001</b>
125 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	<b>&lt; 0.0001</b>

**Table S26: Analysis of *AtHDS* relative transcript abundance after  $\beta$ -cyclocitral treatment in *A. thaliana* plants.** One-way ANOVA showed a significant difference in *AtHDS* relative transcript abundance between untreated and  $\beta$ CC treated plants ( $F_{3,12} = 20.25$ ;  $P < 0.0001$ ). Significance of differences was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Treatments</b>	<b><i>p</i>- value</b>
Water $\times$ 25 $\mu$ l $\beta$ CC	0.9856
Water $\times$ 125 $\mu$ l $\beta$ CC	<b>0.0466</b>
Water $\times$ 250 $\mu$ l $\beta$ CC	<b>0.0027</b>
25 $\mu$ l $\beta$ CC $\times$ 125 $\mu$ l $\beta$ CC	0.0836
25 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	<b>0.0015</b>
125 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	<b>&lt;0.0001</b>

**Table S27: Analysis of *AtHDR* relative transcript abundance after  $\beta$ -cyclocitral treatment in *A. thaliana* plants.** One-way ANOVA showed a significant difference in *AtHDR* relative transcript abundance between untreated and  $\beta$ CC treated plants ( $F_{3,12} = 6.927$ ;  $P = 0.0058$ ). Significance of differences was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Treatments</b>	<b><i>p</i>- value</b>
Water $\times$ 25 $\mu$ l $\beta$ CC	0.0659
Water $\times$ 125 $\mu$ l $\beta$ CC	0.1687
Water $\times$ 250 $\mu$ l $\beta$ CC	0.6709
25 $\mu$ l $\beta$ CC $\times$ 125 $\mu$ l $\beta$ CC	0.9386
25 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	<b>0.0089</b>
125 $\mu$ l $\beta$ CC $\times$ 250 $\mu$ l $\beta$ CC	<b>0.0244</b>

**Table S28: Analysis of *Spodoptera littoralis* larval mass fed on artificial diet supplemented with  $\beta$ -cyclocitral.** One-way ANOVA showed no significant difference in the mass of *S. littoralis* larvae fed on  $\beta$ -cyclocitral supplemented artificial diet as compared to control. ( $F_{3,24}=0.296$ ;  $P=0.828$ ). Significance of differences was determined by Tukey's HSD *post-hoc* test at  $p \leq 0.05$ .

<b>Tukey's HSD <i>post hoc</i></b>	
<b>Treatments</b>	<b><i>p</i>- value</b>
Water $\times$ 0.5 $\mu\text{g g}^{-1}$ $\beta\text{CC}$	0.8412
Water $\times$ 1.0 $\mu\text{g g}^{-1}$ $\beta\text{CC}$	0.9558
Water $\times$ 2.0 $\mu\text{g g}^{-1}$ $\beta\text{CC}$	0.9997
0.5 $\mu\text{g g}^{-1}$ $\beta\text{CC}$ $\times$ 1.0 $\mu\text{g g}^{-1}$ $\beta\text{CC}$	0.9888
1.0 $\mu\text{g g}^{-1}$ $\beta\text{CC}$ $\times$ 2.0 $\mu\text{g g}^{-1}$ $\beta\text{CC}$	0.881
1.0 $\mu\text{g g}^{-1}$ $\beta\text{CC}$ $\times$ 2.0 $\mu\text{g g}^{-1}$ $\beta\text{CC}$	0.9743

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