# Supporting Information

Article title: Salicylic acid activates poplar defense against the biotrophic rust fungus *Melampsora larici-populina via* increased biosynthesis of catechin and proanthocyanidins

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The following Supporting Information is available for this article:

**Table S1** Primer sequences used in this study

| Primer name | Purpose | Primer sequence (5' → 3') | References |
|---|---|---|---|
| WRKY18-for | qPCR | TTATGAAGGAGAGCACAACC | Jiang *et al.*, 2014 |
| WRKY18-rev | qPCR | TTCTGATGGATGATGGACTG | Jiang *et al.*, 2014 |
| WRKY23-for | qPCR | TGCCATGCCAATGCAAAGGAG | Levée *et al.*, 2009 |
| WRKY23-rev | qPCR | ACCAAAACCCAAAGGCGACAAG | Levée *et al.*, 2009 |
| WRKY70-for | qPCR | AATCCAAGGAGCTACTAC | Jiang *et al.*, 2014 |
| WRKY70-rev | qPCR | GTTACCATTGTTGTTGTGG | Jiang *et al.*, 2014 |
| WRKY89-for | qPCR | TCCAACGATCCACAATAACC | Jiang *et al.*, 2014 |
| WRKY89-rev | qPCR | TAAAACATCACCGCCGTCTC | Jiang *et al.*, 2014 |
| NPR1-for | qPCR | GTTGACCTAAATGAGACACC | Jiang *et al.*, 2014 |
| NPR1-rev | qPCR | TAATCTCAGCCTTGTCCTTG | Jiang *et al.*, 2014 |
| PR1-rev | qPCR | TGGGTTGATGAGAAACCAAAGTATG | Hamel *et al.*, 2011 |
| PR1-for | qPCR | GCTGCACCTTGCTTTAGCAC | Hamel *et al.*, 2011 |
| PR2.3-rev | qPCR | CAAAGGATTGCTTCCAGTCAAGC | Jiang *et al.*, 2014 |
| PR2.3-for | qPCR | TCAAGAAGGGCATCGAAGAGG | Jiang *et al.*, 2014 |
| JAZ10a-for | qPCR | CCCCCTTGACTATTTTCTACAACGG | Hamel *et al.*, 2011 |
| JAZ10a-rev | qPCR | GATCTCCATCAAGACTCTCAAGAAGC | Hamel *et al.*, 2011 |
| MYB115-for | qPCR | GCCATTGGAGGTCTTTGCC | Yoshida *et al.*, 2015 |
| MYB115-rev | qPCR | GGTTACCGAGGAGGGAGTGC | Yoshida *et al.*, 2015 |
| MYB134-for | qPCR | CACCACCACCAATACTGCCAC | Ullah *et al.*, 2017 |
| MYB134-rev | qPCR | CCTGGGCTCTTCAGTTCCG | Ullah *et al.*, 2017 |
| MYB182-for | qPCR | GAATCTTTGGTGACACAGCAAGC | Yoshida *et al.*, 2015 |
| MYB182-rev | qPCR | GAAGCAGAGTTGGCAATGATGA | Yoshida *et al.*, 2015 |
| bHLH131-for | qPCR | GTCGATAATAGAGAGTGACGCA | Yoshida *et al.*, 2015 |
| bHLH131-rev | qPCR | CTCTTCACCTCCACAATGCT | Yoshida *et al.*, 2015 |
| CHS1-for | qPCR | TGTGTGAATACATGGCTCCGTCTCT | Yoshida *et al.*, 2015 |
| CHS1-rev | qPCR | GGATTTTGGCTGACCCCACTCTT | Yoshida *et al.*, 2015 |
| CHS4-for | qPCR | TCACTGTTGAGACTGTGGTG | Wang *et al.*, 2017 |
| CHS4-rev | qPCR | CACTCCTTATTGGTGCTCTC | Wang *et al.*, 2017 |
| CHI1-for | qPCR | TGTGCTAGAGTCAATGATTGG | Wang *et al.*, 2017 |
| CHI1-rev | qPCR | GGAAAAGCTTGAGCCTGAAAT | Wang *et al.*, 2017 |

| | | | |
|---|---|---|---|
| F3H1-for | qPCR | TGGTCTGACTTTACAACGTGC | Wang *et al.,* 2017 |
| F3H1-rev | qPCR | GACAACTCACACGGCATTGC | Wang *et al.,* 2017 |
| DFR1-for | qPCR | CTTATAACTGCCCTTTCTCTGA | Yoshida *et al.,* 2015 |
| DFR1-rev | qPCR | AGATCATGAATGGTGGCTT | Yoshida *et al.,* 2015 |
| PnLAR1-for | qPCR | CGAGTACTCATAGCCGGAGC | Ullah *et al.,* 2017 |
| PnLAR1-rev | qPCR | GGCTCCTTTGTCGTGAAGAG | Ullah *et al.,* 2017 |
| PnLAR2-for | qPCR | AACAAGTCGGTCCATTTTCG | Ullah *et al.,* 2017 |
| PnLAR2-rev | qPCR | GCA GCAATAGCAAGGAGGTC | Ullah *et al.,* 2017 |
| PnLAR3-for | qPCR | GAAGCTAGCCTCGAATGTGG | Ullah *et al.,* 2017 |
| PnLAR3-rev | qPCR | TTGGTCTGCTATGCTTGCAC | Ullah *et al.,* 2017 |
| PnANR1-for | qPCR | GCATCCCAGACCAAGAAAAA | Ullah *et al.,* 2017 |
| PnANR1-rev | qPCR | TCCCCCAAATTCTGTAGTGC | Ullah *et al.,* 2017 |
| PnANR2-for | qPCR | CCTGCCTCCAAGACACTAGC | Ullah *et al.,* 2017 |
| PnANR2-rev | qPCR | GCTGCTGGGAATATCTAGCG | Ullah *et al.,* 2017 |
| Ubiquitin-for | qPCR | GTTGATTTTTGCTGGGAAGC | Irmisch *et al.*, 2013 |
| Ubiquitin-rev | qPCR | GATCTTGGCCTTCACGTTGT | Irmisch *et al.*, 2013 |
| MlpActin-for | qPCR | GACTGAGGCACCTCTTAATCCAAAAGTC | Ullah *et al.,* 2017 |
| MlpActin-rev | qPCR | GTGAGTAACACCGTCACCAGAATCC | Ullah *et al.,* 2017 |
| WD40-for | qPCR | CTCGCACGAACCAATACCAG | This study |
| WD40-rev | qPCR | AACATGAGCTTTGTGGGTGG | This study |

## References


**Hamel LP, Benchabane M, Nicole MC, Major IT, Morency MJ, Pelletier G, Beaudoin N, Sheen J, Seguin A. 2011.** Stress-responsive mitogen-activated protein kinases interact with the EAR motif of a poplar zinc finger protein and mediate its degradation through the 26S proteasome. *Plant Physiology* **157**(3): 1379-1393.

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**Jiang Y, Duan Y, Yin J, Ye S, Zhu J, Zhang F, Lu W, Fan D, Luo K. 2014.** Genome-wide identification and characterization of the Populus WRKY transcription factor family and analysis of their expression in response to biotic and abiotic stresses. *Journal of Experimental Botany* **65**(22): 6629-6644.

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**Ullah C, Unsicker SB, Fellenberg C, Constabel CP, Schmidt A, Gershenzon J, Hammerbacher A. 2017.** Flavan-3-ols are an effective chemical defense against rust infection. *Plant Physiology* **175**(4): 1560-1578.

**Wang L, Ran L, Hou Y, Tian Q, Li C, Liu R, Fan D, Luo K. 2017.** The transcription factor MYB115 contributes to the regulation of proanthocyanidin biosynthesis and enhances fungal resistance in poplar. *New Phytologist* **215**(1): 351-367.

**Yoshida K, Ma D, Constabel CP. 2015.** The MYB182 Protein Down-Regulates Proanthocyanidin and Anthocyanin Biosynthesis in Poplar by Repressing Both Structural and Regulatory Flavonoid Genes. *Plant Physiology* **167**(3): 693-710.

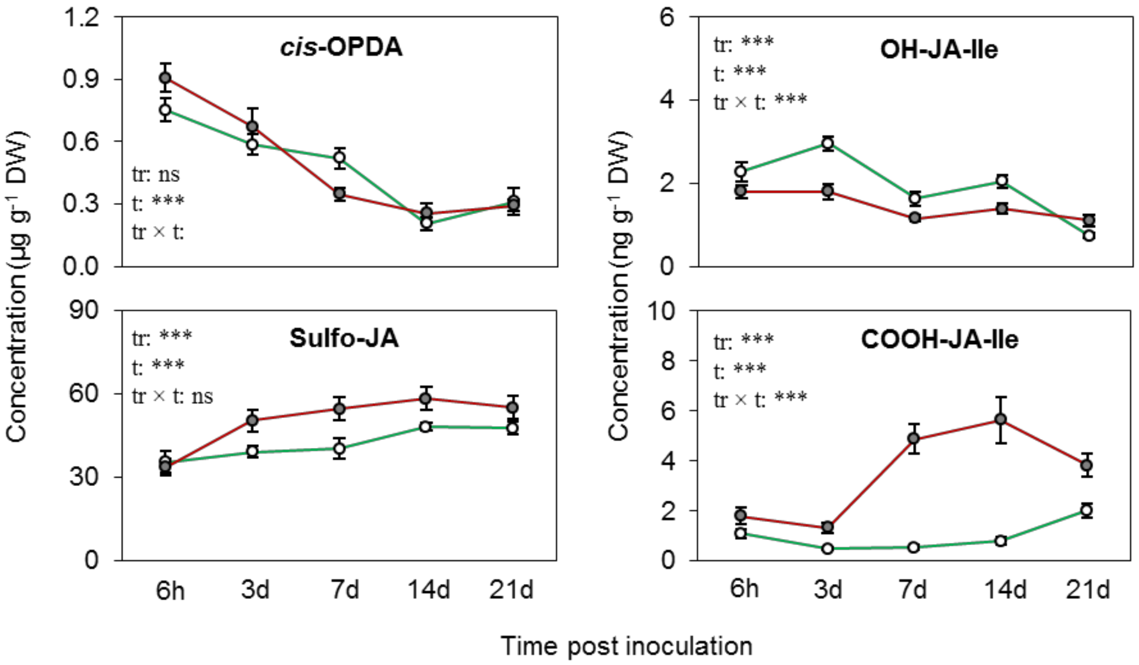


**Fig. S1** Effect of rust infection on the concentrations of the jasmonic acid (JA) precursor, *cis*-OPDA, and JA catabolites in black poplar leaves over the course of infection. Rust-infected trees are shown by red lines (closed circles) and non-infected control trees are shown by green lines (open circles). *cis*-OPDA = *cis*-12-oxo-phytodienoic acid, Sulfo-JA = 12-sulfojasmonic acid, OH-JA-Ile = 12-hydroxyjasmonic acid isoleucine, COOH-JA-Ile = 12-carboxyjasmonic acid isoleucine. Data were analyzed by two-way ANOVA. Data are expressed as the mean ± SE (n=5), and each replicate was a pool of six fully expanded leaves (LPI 5-10) from a single tree. tr = treatment; t = time; **, $p \leq 0.01$; and ***, $p \leq 0.001$; ns, non-significant; h = hour, d = day, DW = dry weight.

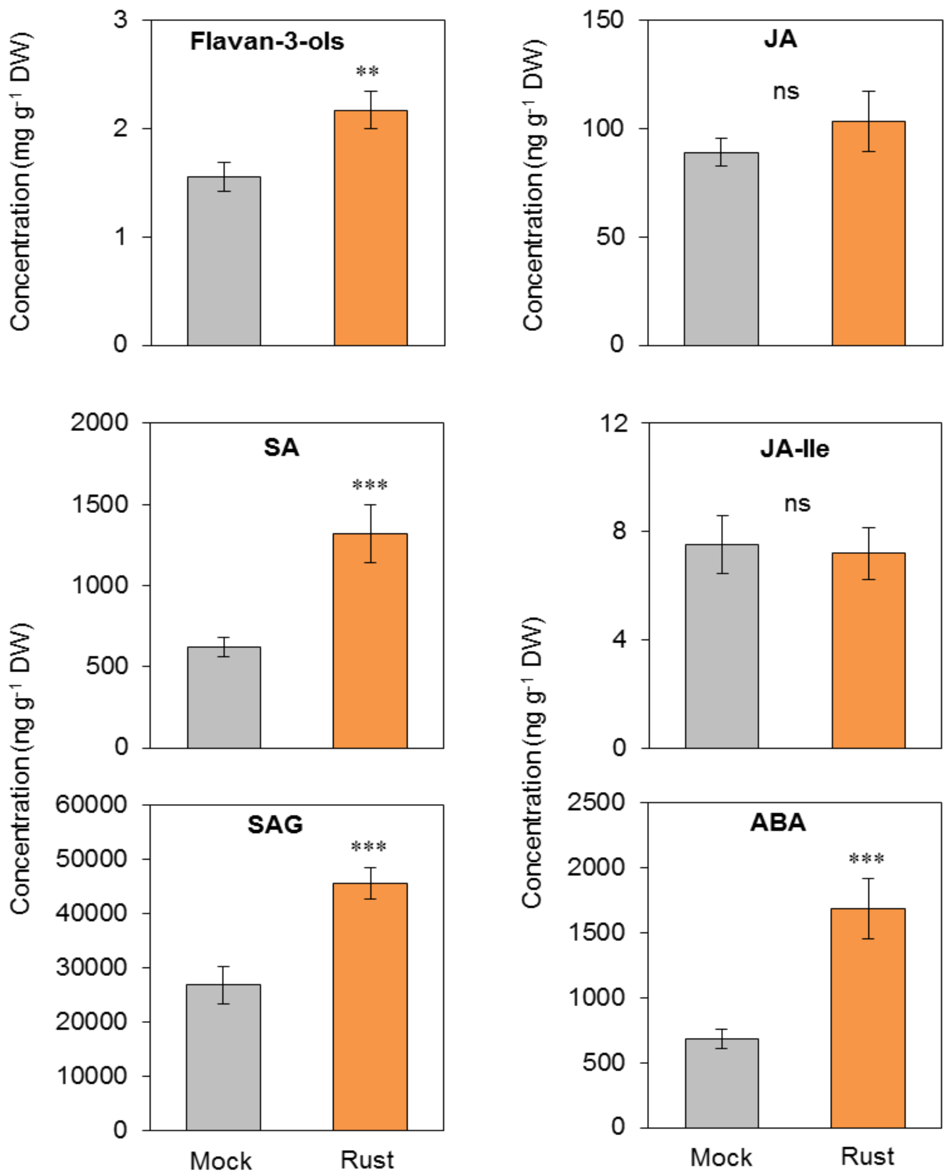


**Fig. S2** Effect of rust infection on the accumulation of flavan-3-ols, abscisic acid and salicylic acid in expanding systemic leaves of black poplar trees. Growing shoots (leaf 1-5 counted basipetally) were covered with polyethylene terephthalate (PET) bags before rust inoculation (Rust) or spraying with water (Mock) to protect from fungal infection. Young expanding leaves (LPI 1-5) without rust symptoms were sampled 7 days after inoculation. Flavan-3-ols were measured as the sum of catechin, epicatechin, gallocatechin and proanthocyanidin dimers. SA = salicylic acid, SAG = salicylic acid glucoside, ABA = abscisic acid, JA = jasmonic acid, JA-Ile = jasmonic acid isoleucine, DW = dry weight. Data were analyzed by Student's t-test (**, $p < 0.01$; ***, $p < 0.001$; ns, non-significant). Bars represent mean ± SE (n=5).

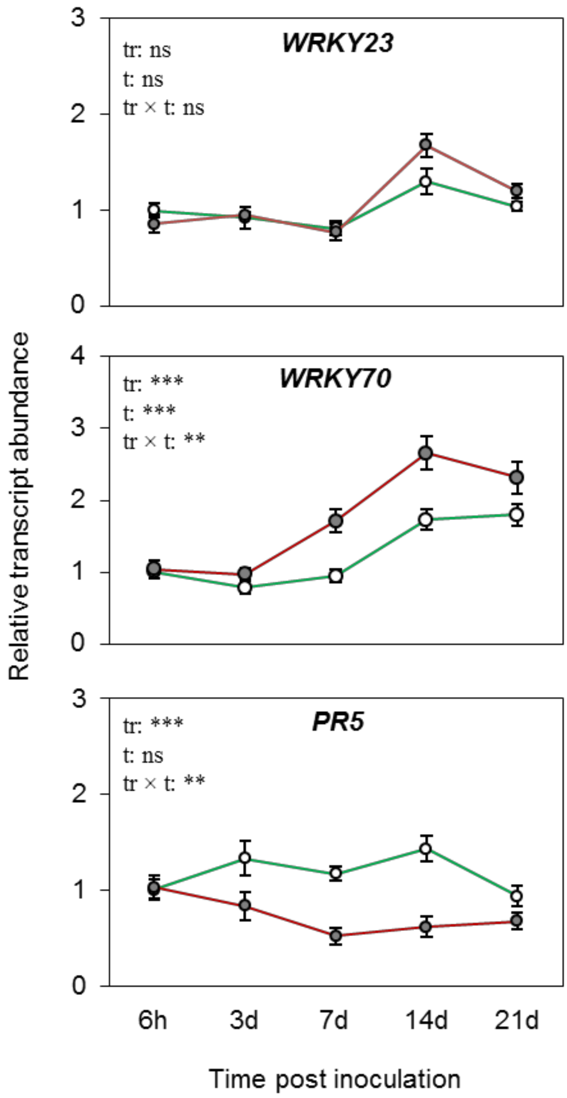


**Fig. S3** Effect of rust infection on the relative expression levels of *WRKY23*, *WRKY70* and *PR5*. Rust-infected trees are shown by red lines (closed circles) and non-infected control trees are shown by green lines (open circles). Transcript levels of each gene were normalized to *ubiquitin* transcripts. Three technical replicates were used per sample during qRT-PCR. Data were analyzed by two-way ANOVA (Factors: "tr" = treatment, "t" = time post inoculation). Data are expressed as the mean ± SE (n=5), and each replicate was a pool of six fully expanded leaves (LPI 5-10) from a single tree. tr = treatment; t = time; **, $p \leq 0.01$; and ***, $p \leq 0.001$; ns, non-significant. h = hour; d = day.

**Table S2** Levels of phenolic metabolites in black poplar leaves one day after exogenous hormone treatment.

| Phenolic metabolites | Treatment | | | |
|---|---|---|---|---|
| | Mock | BTH | MeJA | ABA |
| Catechin (µg/g) | 293 ± 21 | 441 ± 46 | 350 ± 55 | 337 ± 58 |
| Epicatechin (µg/g) | 19 ± 5.2 | 28 ± 3.1 | 22 ± 5.3 | 19 ± 4.6 |
| Gallocatechin (µg/g) | 46 ± 8.3 | 50 ± 4.1 | 66 ± 14.7 | 39 ± 4.0 |
| PAB1 (µg/g) | 91 ± 11 | 124 ± 17 | 89 ± 15 | 95 ± 19 |
| Salicinoids (mg/g) | 34 ± 1.2 | 31 ± 1.4 | 33 ± 1.6 | 33 ± 1.7 |
| Phenolic acids (µg/g) | 8.3 ± 0.40 a | 6.0 ± 0.39 b | 6.8 ± 0.31 ab | 8.1 ± 0.42 a |
| Rutin (mg/g) | 5.1 ± 0.97 | 5.9 ± 0.87 | 5.4 ± 0.81 | 5.5 ± 0.79 |

Leaf laminae (LPI 5-10) from benzothiadiazole (BTH), methyl jasmonate (MeJA), abscisic acid (ABA) and mock-treated young black poplar trees were analyzed one day after treatment, just before rust inoculation (0 dpi). PAB1 = procyanidin B1. Salicinoids were the sum of salicin, salicortin and homaloside D. Phenolic acids were the sum of cinnamic acid, coumaric acid, caffeic acid and ferulic acid. Data were analyzed by one-way ANOVA followed by Tukey's Post-hoc test. Different letters indicate groups were statistically different ($p < 0.05$). Data are presented as the mean ± SE (n=4).

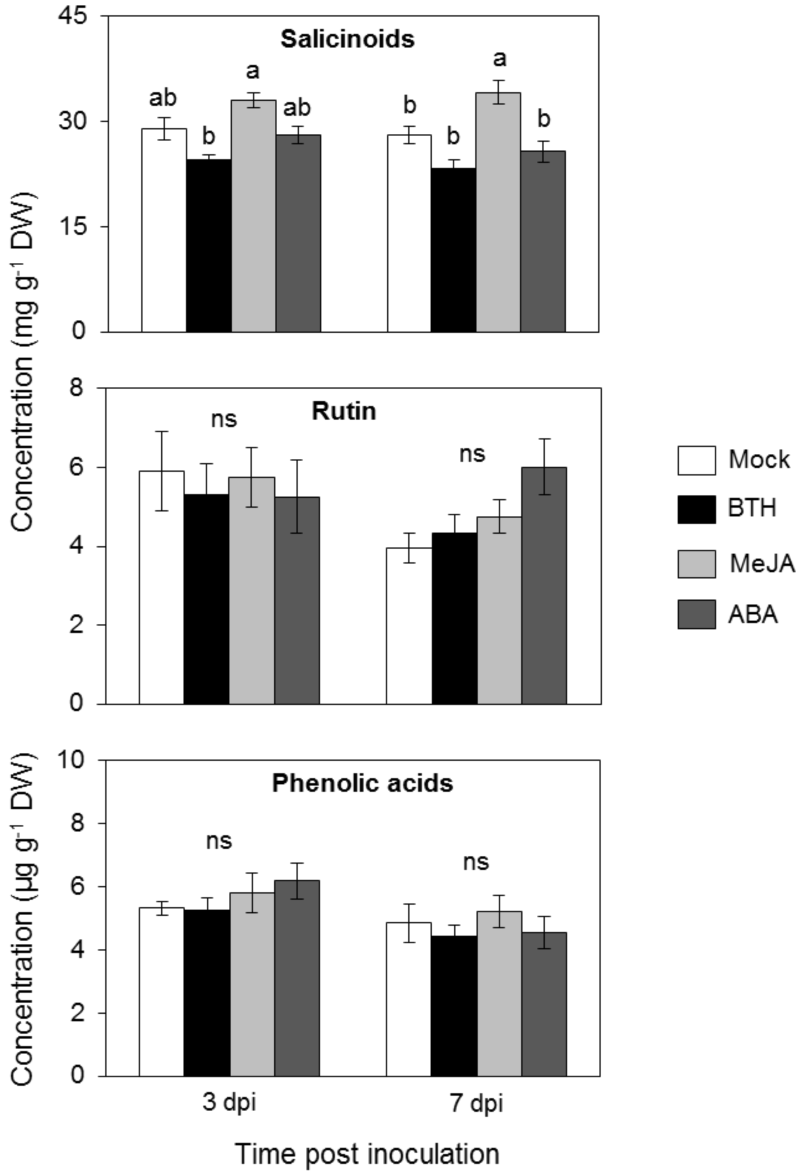


**Fig. S4** Phytohormone application before rust infection did not alter the levels of other phenolic metabolites in poplar besides flavan-3-ols. Salicinoids were the sum of salicin, salicortin and homaloside D. Phenolic acids were the sum of cinnamic acid, coumaric acid, caffeic acid and ferulic acid. Samples were collected from separate trees at each time point for all treatments. Time-course data were analyzed by one-way ANOVA followed by Tukey's Post-hoc test. Different letters indicate groups were statistically different ($p < 0.05$). Data are presented as the mean ± SE (n=5), and each replicate was a pool of six fully expanded leaves (LPI 5-10) from a single tree. dpi = day post inoculation of rust fungus, ns = non-significant, DW = dry weight.

**Table S3** Levels of endogenous hormones in black poplar leaves one day after exogenous hormone treatment.

| Metabolites | Treatment | | | |
|---|---|---|---|---|
| | Mock | BTH | MeJA | ABA |
| SA (ng/g) | 325 ± 76 ab | 213 ± 38 b | 649 ± 129 a | 517 ± 71 a |
| JA (ng/g) | 70 ± 14 b | 50 ± 12 b | 1970 ± 141 a | 82 ± 14 b |
| JA-Ile (ng/g) | 5.1 ± 0.9 b | 5.7 ± 0.7 b | 34.7 ± 4.5 a | 5.8 ± 0.8 b |
| *cis*-OPDA (ng/g) | 517 ± 55 b | 425 ± 43 b | 1295 ± 69 a | 274 ± 25 c |
| OH-JA (ng/g) | 498 ± 70 b | 362 ± 46 b | 2962 ± 594 a | 275 ± 44 b |
| OH-JA-Ile (ng/g) | 3.89 ± 0.38 b | 3.15 ± 0.43 b | 44.79 ± 5.84 a | 4.07 ± 0.63 b |
| COOH-JA-Ile (ng/g) | 1.34 ± 0.40 b | 1.74 ± 0.39 b | 10.13 ± 0.78 a | 1.77 ± 0.28 b |
| ABA (ng/g) | 79 ± 15 c | 76 ± 20 c | 349 ± 78 b | 37236 ± 3287 a |

Leaf laminae (LPI 5-10) from benzothiadiazole (BTH), methyl jasmonate (MeJA), abscisic acid (ABA) and mock-treated young black poplar trees were analyzed one day after treatment, just before rust inoculation (0 dpi). SA = salicylic acid, JA = jasmonic acid, JA-Ile = jasmonic acid-isoleucine conjugate, *cis*-OPDA = *cis*-12-oxo-phytodienoic acid, OH-JA = 12-hydroxyjasmonic acid, COOH-JA-Ile = 12-carboxyjasmonic acid-isoleucine, OH-JA-Ile = 12-hydroxyjasmonic acid-isoleucine. Data were analyzed by one-way ANOVA followed by Tukey's Post-hoc test. Different letters indicate significant differences between treatments ($p < 0.05$). Data are presented as the mean ± SE (n=4).

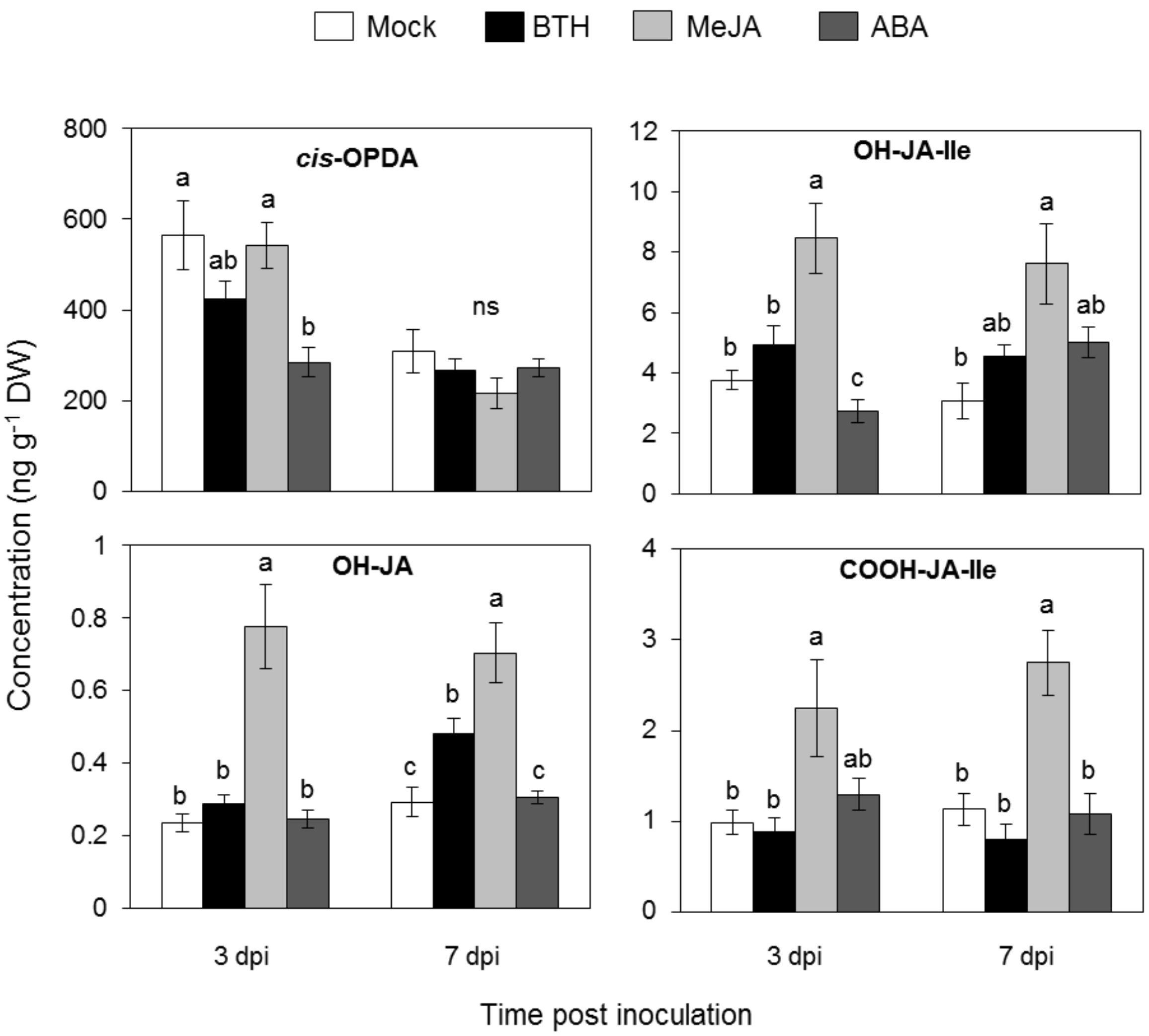


**Fig. S5** Jasmonate concentrations in *Populus nigra* leaves after exogenous phytohormone application followed by rust infection. *cis*-OPDA = *cis*-12-oxo-phytodienoic acid, OH-JA = 12-hydroxyjasmonic acid, OH-JA-Ile = 12-hydroxyjasmonic acid-isoleucine, COOH-JA-Ile = 12-carboxyjasmonic acid-isoleucine. Samples were collected from separate trees at each time point for all treatments. Time-course data were analyzed by one-way ANOVA followed by Tukey's Post-hoc test. Different letters indicate groups were statistically different at 95% confidence. Data are presented as the mean ± SE (n=5), and each replicate was a pool of six fully expanded leaves (LPI 5-10) from a single tree. dpi = day post inoculation of rust fungus; ns = non-significant, DW = dry weight.

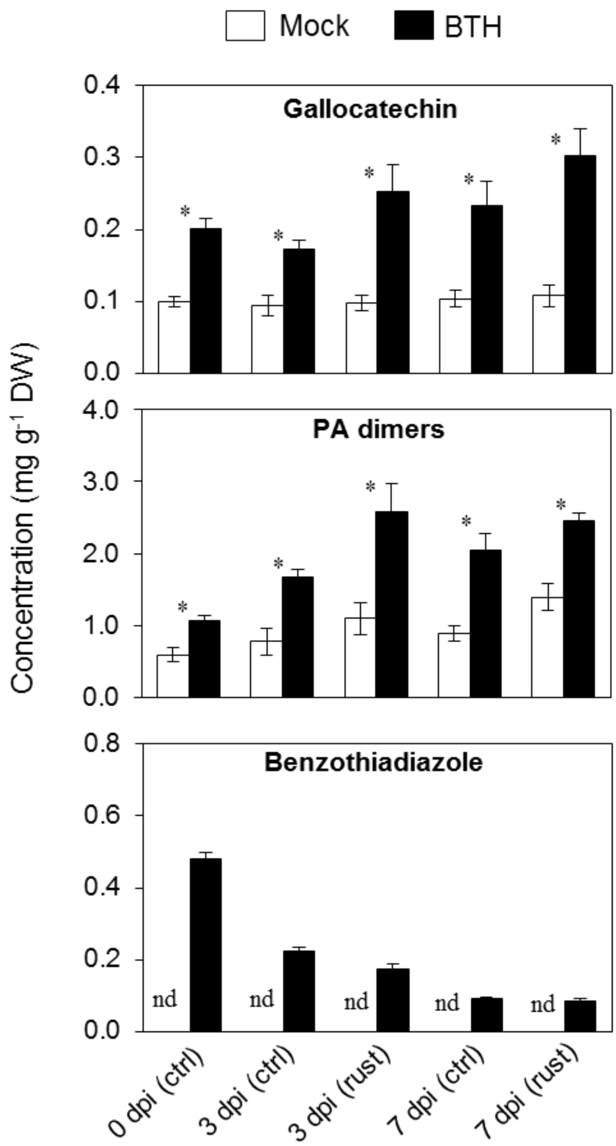


**Fig. S6** Accumulation of flavan-3-ols in black poplar leaves treated with the salicylic acid analogue benzothiadiazole. Data (mock *vs* BTH) were analyzed by Student's t-test (*, $p < 0.05$). Data are presented as the mean ± SE (n=4), and each replicate was a pool of six fully expanded leaves (LPI 5-10) from a single tree. PA = proanthocyanidins, dpi = day post inoculation, ctrl= water-treated control, rust = rust-inoculated, nd = not detected, DW = dry weight.

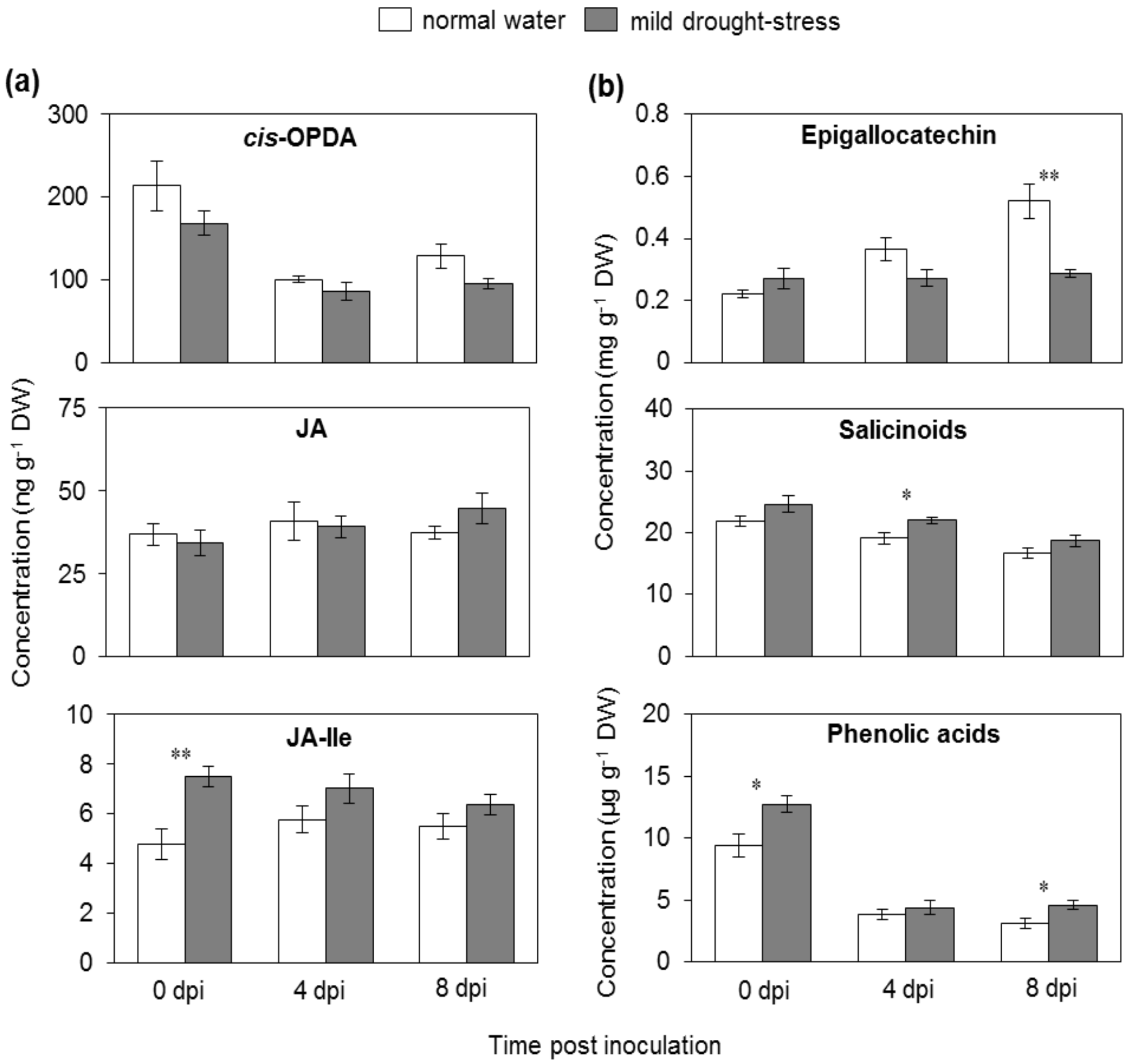


**Fig. S7** Concentrations of jasmonates, epigallocatechin, salicinoids and phenolic acids in poplar (*Populus* × *canadensis* Robusta) leaves under mild drought stress and rust infection. Young poplar trees were watered normally or exposed to mild drought stress. After 7 days, a subset of plants was sampled (0 dpi) and the remaining trees were inoculated with *M. larici-populina*. The drought treatment was continued until the end of the experiment. *cis*-OPDA = *cis*-12-oxo-phytodienoic acid, JA = jasmonic acid, JA-Ile = jasmonic acid isoleucine. Salicinoids were the sum of salicin, salicortin and homaloside D. Phenolic acids were the sum of cinnamic acid, coumaric acid, caffeic acid and ferulic acid. Data were analyzed by Student's t-test (*, $p < 0.05$ and **, $p \leq 0.01$). Data are presented as the mean ± SE (n=5), and each replicate was a pool of five fully expanded leaves (LPI 5-10) from a single tree. dpi = days post inoculation, ns = non-significant, DW = dry weight.