Supporting information

Flowers prepare thyselves:
Leaf and root herbivores induce specific changes in floral phytochemistry with consequences for plant interactions with florivores.

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Methods S1

Phytohormone analysis

Phytohormone analysis was performed by LC-MS/MS as in Vadassery *et al.* (2012) on an Agilent 1200 series HPLC system (Agilent Technologies) with the modification that a tandem mass spectrometer QTRAP 6500 (SCIEX, Darmstadt, Germany) was used. Separation of raw extracts was achieved on a Zorbax Eclipse XDB-C18 column (50 × 4.6 mm, 1.8 mm; Agilent) with a solvent system of 0.05% formic acid (A) and acetonitrile (B) at a flow rate of 1.1 ml/min. The elution profile was the following: 0 to 0.5 min, 10% B; 0.5 to 4.0 min, 10% to 90% B in A; 4.0 to 4.02 min, 90% to100% B, 4.02 to 4.5 min, 100% B and 4.51 to 7.0 min 10% B. Electrospray ionization (ESI) in negative ionization mode was used for the coupling of LC to MS. The mass spectrometer parameters were set as follows: ion spray voltage, -4500 V; turbo gas temperature, 700 °C; collision gas, 7 psi; curtain gas, 35 psi; ion source gas 1, 60 psi; ion source gas 2, 60 psi. Parent ion to product ion was monitored by multiple reaction monitoring (MRM) as follows: m/z 136.9 →93.0 (collision energy [CE], -24 V; declustering potential [DP], -40 V) for SA; m/z 140.9 →97.0 (CE, -24 V; DP, -40 V) for D4-SA; m/z 290.9 → 165.1 (CE, -24 V; DP, -45 V) for cis-OPDA; m/z 209.1 →59.0 (CE -24 V; DP -35 V) for JA; m/z 225.1 →59.0 (CE, -24 V; DP, -35 V) for OH-JA; m/z 322.2 →130.1 (CE, -30 V; DP, -50 V) for JA-Ile; m/z 338.1 →130.1 (CE, -30 V; DP, -50 V) for OH-JA-Ile; m/z 352.1 →130.1 (CE, -30 V; DP, -50 V) for COOH-JA-Ile; m/z 263.0 →153.2 (CE, -22 V; DP, -35 V) for ABA; m/z 269.0 →159.2 (CE, -22 V; DP, -35 V) for D6-ABA. Since it was observed that both the D6-labeled JA and D6-labeled JA-Ile standards (HPC Standards GmbH, Cunnersdorf, Germany) contained 40% of the corresponding D5-labeled compounds, the sum of the peak areas of D5- and D6-compound was used for quantification: sum m/z 215.1 →59.0 +

m/z 214.1 →59.0 (CE -24 V; DP -35 V) for D6-JA; sum m/z 328.2 →130.1+ m/z 327.2 →130.1 (CE, -30 V; DP, -50 V) for D6-JA-Ile. Data processing was performed using Analyst 1.5 software and analyte quantity was determined relative to the corresponding internal standard peak area. Concentration of cis-OPDA, and OH-JA were determined relative to the quantity of the internal standard D6-JA with a theoretical response factor one. OH-JA-Ile and COOH-JA-Ile were quantified relative to D6-JA-Ile with a theoretical response factor one.

Statistical analyses

Model assumptions for linear (mixed) models (linearity, normality, and homogeneity of variance) were assessed graphically using plots of deviance residuals against fitted values by the models as well as against all the included fixed and random factors, and a QQ-plot. Model assumptions, *i.e.* overdispersion, for generalized linear models were tested using the dispersiontest() function from the AER package (Kleiber & Zeileis, 2008). For generalized linear mixed models, we used the overdisp_fun function (Bolker, 2021). All lm/lmer/glm/glmer's were performed with respective functions from the lme4 package (Bates *et al.*, 2015). P-values were derived with the lrtest function from the lmtest package (Zeileis & Hothorn, 2002). Random factors were selected using a backward approach; all random factors were initially added to the model and removed if they explained less than 5% of the variation or were statistically non-significant ($P > 0.05$). Tukey's post-hoc tests were performed using the glht function (one fixed factor; multcomp package (Hothorn *et al.*, 2008)) and emmeans function (2 fixed factors with interactive effect; lsmeans package (Lenth, 2016).

PERMANOVA analyses were performed with the vegan package (Oksanen *et al.*, 2019), and pairwise comparisons for all pairs of levels of a factor (PERMANOVA-PAIR) were performed with the EcolUtils package (Salazar, 2020). Data was range scaled using the normalize function from the effectsize package (Ben-Shachar *et al.*, 2020). Project to latent structures discriminant (PLS-DA) analysis was performed with the mixOmics package (Rohart *et al.*, 2017).

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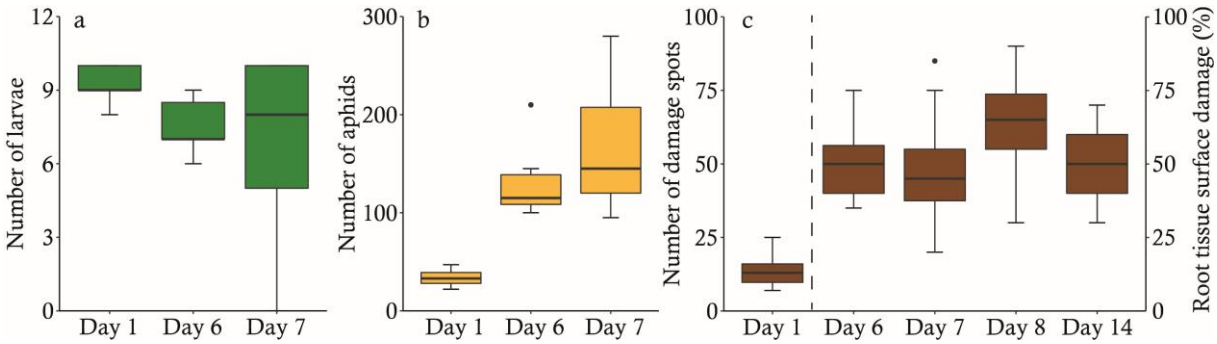

Figure S1. Treatment intensities for *Brassica nigra* plants damaged by leaf and root herbivores measured on different timepoints since herbivore infestation. a) Number of larvae counted and removed from plants damaged by *Athalia rosae*. b) Number of aphids counted and removed from plants damaged by *Lipaphis erysimi*. c) Root damage assessed for plants damaged by *Delia radicum*. For day 1, we counted the number of damage spots. For other days, we estimated the percentage of root tissue (tap- and lateral roots) surface damage. Boxplots show median (line), 1st and 3rd quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the 1st or above the 3rd quartile) are represented by circles.

Table S1. Output of post-hoc analyses for models of preferences of adult *Pieris brassicae* butterflies and winged *Brevicoryne brassicae* aphids for damaged and undamaged *Brassica nigra* plant pairs. Plants were damaged for 7 days by one of three herbivores: *Athalia rosae*, *Lipaphis erysimi*, or *Delia radicum*, and paired with an undamaged plant. E = estimate, SE = standard error. Bold values indicate results where $P \leq 0.05$. Italic values indicate results where $P \leq 0.1$.

	Athalia rosae-damaged VS Uninfested				*Lipaphis erysimi*-damaged VS Uninfested				*Delia radicum*-damaged VS Uninfested			
Butterfly preference	E	SE	z	*P*	E	SE	z	*P*	E	SE	z	P
Landing choice	0.20	0.22	0.89	0.372	-0.35	0.22	-1.60	0.110	0.71	0.24	2.97	**0.003**
Oviposition choice	-0.15	0.22	-0.67	0.503	-0.49	0.22	-2.23	**0.026**	0.73	0.34	2.12	**0.034**
Total time spent	-0.35	0.22	-1.55	0.122	-0.28	0.22	-1.28	0.202	1.62	0.90	1.80	*0.072*
Oviposition time	-0.20	0.22	-0.92	0.357	-0.31	0.23	-1.33	0.182	1.11	0.58	1.92	*0.055*
Aphid preference	-0.67	0.16	-4.17	**<0.001**	0.66	0.18	3.68	**<0.001**	-0.37	0.15	-2.51	**0.012**

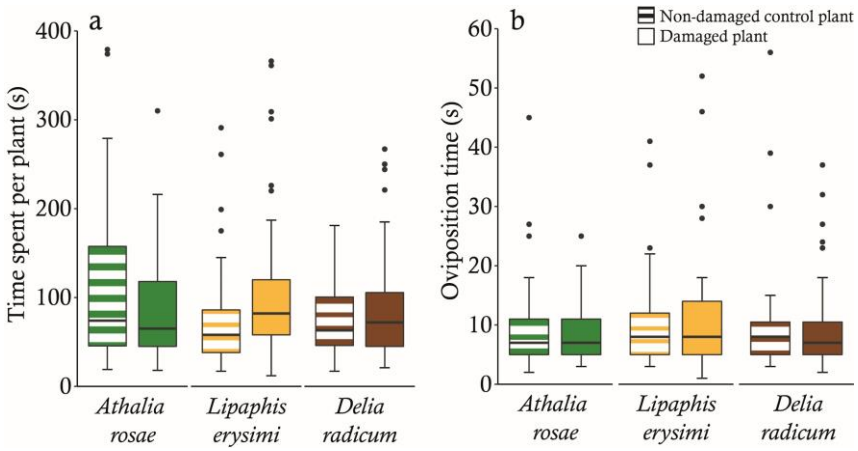

Figure S2. a) Total time spent and b) Oviposition time by *Pieris brassicae* butterflies on *Brassica nigra* plants damaged by different herbivores and undamaged plants. Oviposition time was measured as time between landing on the plant of choice and laying the first egg. Boxplots show median (line), 1st and 3rd quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the 1st or above the 3rd quartile) are represented by circles. Butterfly behaviour was assessed after seven days of herbivory by one of three herbivores. Number of replicates per herbivore treatment varied between 79-87 butterflies, and 8-9 plant pairs.

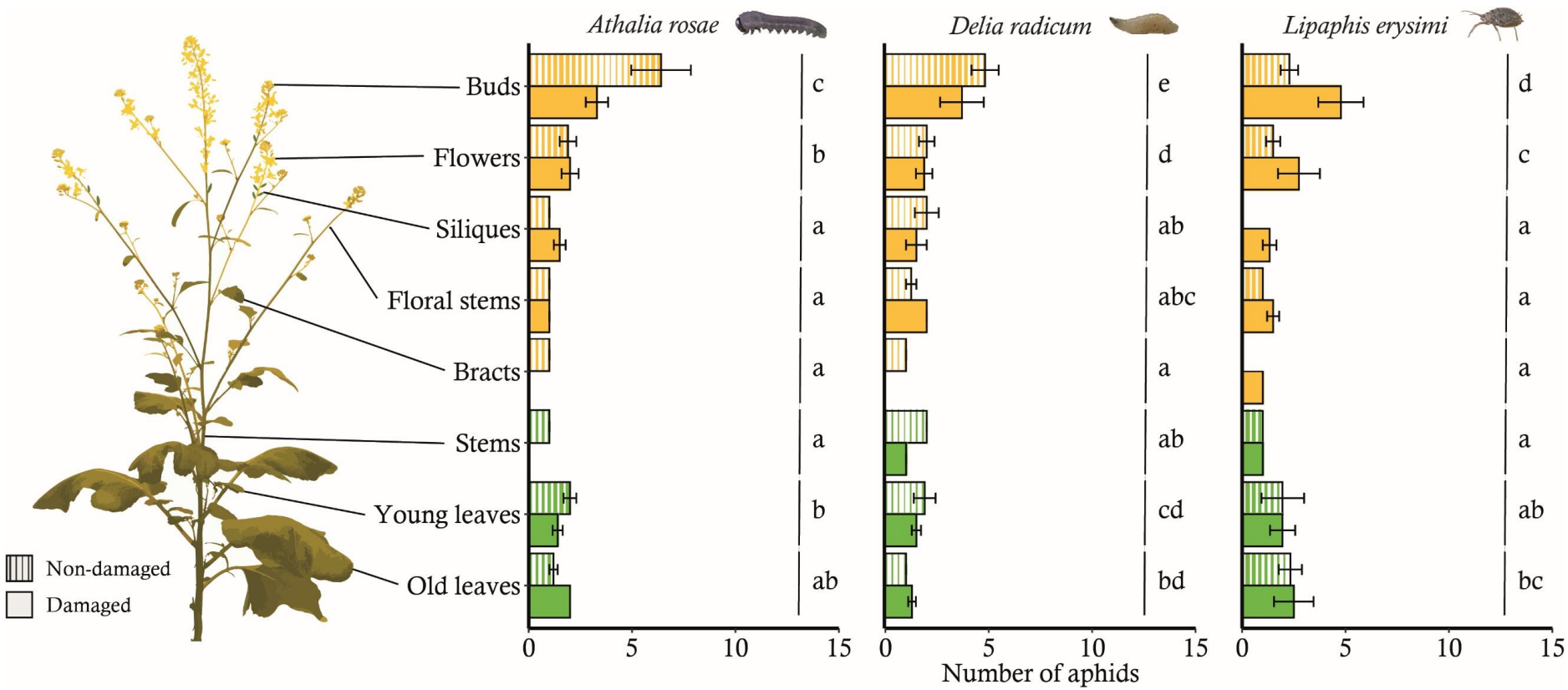

Figure S3. Number of winged aphids (Mean ± SE) on various plant organs of damaged and undamaged *Brassica nigra* plants. The position of 20 winged *Brevicoryne brassicae* aphids was determined 24 h after release. Damaged plants were exposed to one of three herbivores for 7 days. Each herbivore treatment was replicated 9-12 times. Letters indicate significant differences between plant organs with $P \leq 0.05$ based on Tukey's post hoc tests. Photo credits: Jitte Groothuis, Dani Lucas-Barbosa, Quint Rusman.

Table S2. Output of post-hoc analyses for models of *Pieris brassicae* caterpillar performance and phytochemistry of plant tissues (buds and flowers) of *Brassica nigra* plants comparing damaged and undamaged (C) plants. Plants were damaged for 7 days by one of three herbivores: *Athalia rosae* (Ar), *Lipaphis erysimi* (Le), or *Delia radicum* (Dr). E = estimate, SE = standard error, *LOX2* = *LIPOXYGENASE 2*, OPDA = 12-oxo-phytodienoic acid, OH-JA = hydroxy-JA, OH-JA-Ile = hydroxy-JA-Ile, *PR1* = *PATHOGENESIS-RELATED PROTEIN 1*, GLS = glucosinolates, 4OHI3M = 4-hydroxy-glucobrassicin, 2PE = gluconasturtiin, I3M = glucobrassicin. Bold values indicate results where $P \leq 0.05$. Italic values indicate results where $P \leq 0.1$.

	Ar - C				Ar - Dr				Ar - Le				C - Dr				C - Le				Dr - Le			
	E	SE	z/t	*P*	E	SE	z/t	*P*	E	SE	z/t	P	E	SE	z/t	*P*	E	SE	z/t	*P*	E	SE	z/t	*P*
Caterpillar performance	0.32	0.10	3.12	**0.010**	0.67	0.15	4.59	**0.001**	0.02	0.13	0.13	0.999	0.35	0.13	2.66	**0.037**	-0.30	0.11	2.74	**0.030**	-0.66	0.15	-4.30	**0.001**
Phytochemistry buds timepoint 1																								
LOX2	0.00	0.12	0.02	1.000	-0.30	0.13	-2.39	*0.079*	-0.11	0.13	-0.84	0.835	-0.30	0.11	-2.67	**0.038**	-0.11	0.12	-0.95	0.779	0.19	0.12	1.54	0.412
OPDA	-0.70	0.19	-3.67	**0.001**	-1.43	0.20	-7.03	**<0.001**	-0.39	0.21	-1.82	0.264	-0.74	0.19	-3.99	**<0.001**	0.31	0.19	1.62	0.367	1.05	0.21	5.07	**<0.001**
OH-JA	-0.05	0.08	-0.58	0.937	-0.06	0.09	-0.65	0.916	-0.04	0.09	-0.13	0.999	-0.01	0.08	-0.13	0.999	0.01	0.08	0.13	0.999	0.02	0.09	0.23	0.996
OH-JA-Ile	0.17	0.13	1.37	0.518	0.28	0.14	2.00	0.187	0.16	0.14	1.12	0.676	0.10	0.13	0.82	0.843	-0.02	0.13	-0.14	0.999	-0.12	0.14	-0.88	0.815
PR1	2.40	0.56	4.29	**<0.001**	2.94	0.62	4.73	**<0.001**	2.86	0.62	4.61	**<0.001**	0.54	0.56	0.96	0.773	0.46	0.56	0.82	0.843	-0.08	0.62	-0.12	0.999
Total GLS	15.24	4.07	3.74	**0.001**	27.26	4.37	6.23	**<0.001**	17.67	4.54	3.89	**<0.001**	12.03	3.96	3.04	**0.015**	2.43	4.14	0.59	0.936	-9.59	4.43	-2.16	0.138
sinigrin	14.99	3.99	3.76	**0.001**	26.53	4.28	6.19	**<0.001**	17.38	4.44	3.91	**<0.001**	11.55	3.88	2.98	**0.017**	2.39	4.05	0.59	0.935	-9.15	4.34	-2.11	0.155
4OHI3M	0.06	0.10	0.60	0.932	0.417	0.11	3.96	**<0.001**	0.03	0.11	0.30	0.990	0.36	0.10	3.76	**0.001**	-0.03	0.10	-0.26	0.994	-0.38	0.11	-3.60	**0.002**
2PE	0.02	0.11	0.15	0.999	0.37	0.12	3.20	**0.007**	0.29	0.12	2.49	*0.062*	0.35	0.11	3.39	**0.004**	0.27	0.11	2.60	**0.047**	-0.08	0.12	-0.72	0.890
I3M	0.19	0.12	1.56	0.742	0.39	0.13	3.00	**0.048**	0.26	0.13	1.90	0.510	0.20	0.12	1.71	0.644	0.07	0.12	0.53	0.999	-0.14	0.13	-1.06	0.953
Phytochemistry buds timepoint 6																								
LOX2	0.00	0.12	0.02	1.000	-0.30	0.13	-2.39	*0.079*	-0.11	0.13	-0.84	0.835	-0.30	0.11	-2.67	**0.038**	-0.11	0.12	-0.95	0.779	0.19	0.12	1.54	0.412
OPDA	-0.70	0.19	-3.67	**0.001**	-1.43	0.20	-7.03	**<0.001**	-0.39	0.21	-1.82	0.264	-0.74	0.19	-3.99	**<0.001**	0.31	0.19	1.62	0.367	1.05	0.21	5.07	**<0.001**
OH-JA	-0.05	0.08	-0.58	0.937	-0.06	0.09	-0.65	0.916	-0.04	0.09	-0.13	0.999	-0.01	0.08	-0.13	0.999	0.01	0.08	0.13	0.999	0.02	0.09	0.23	0.996
OH-JA-Ile	1.85	0.13	14.53	**<0.001**	1.35	0.13	10.17	**<0.001**	2.04	0.14	14.70	**<0.001**	-0.49	0.12	-4.08	**<0.001**	0.20	0.13	1.57	0.399	0.69	0.13	5.18	**<0.001**
PR1	0.24	0.57	0.43	0.974	1.72	0.60	2.89	**0.020**	0.17	0.62	0.28	0.993	1.47	0.54	2.74	**0.031**	-0.07	0.57	-0.13	0.999	-1.55	0.60	-2.60	**0.046**
Total GLS	15.24	4.07	3.74	**0.001**	27.26	4.37	6.23	**<0.001**	17.67	4.54	3.89	**<0.001**	12.03	3.96	3.04	**0.015**	2.43	4.14	0.59	0.936	-9.59	4.43	-2.16	0.138
sinigrin	14.99	3.99	3.76	**0.001**	26.53	4.28	6.19	**<0.001**	17.38	4.44	3.91	**<0.001**	11.55	3.88	2.98	**0.017**	2.39	4.05	0.59	0.935	-9.15	4.34	-2.11	0.155
4OHI3M	0.06	0.10	0.60	0.932	0.417	0.11	3.96	**<0.001**	0.03	0.11	0.30	0.990	0.36	0.10	3.76	**0.001**	-0.03	0.10	-0.26	0.994	-0.38	0.11	-3.60	**0.002**
2PE	0.41	0.11	3.91	**<0.001**	0.40	0.11	3.60	**0.002**	0.31	0.12	2.58	**0.049**	-0.01	0.10	-0.13	0.999	-0.11	0.11	-0.97	0.767	-0.09	0.11	-0.81	0.850
I3M	0.19	0.12	1.56	0.742	0.39	0.13	3.00	**0.048**	0.26	0.13	1.90	0.510	0.20	0.12	1.71	0.644	0.07	0.12	0.53	0.999	-0.14	0.13	-1.06	0.953
Phytochemistry flowers timepoint 1																								
LOX2	0.00	0.12	0.02	1.000	-0.30	0.13	-2.39	*0.079*	-0.11	0.13	-0.84	0.835	-0.30	0.11	-2.67	**0.038**	-0.11	0.12	-0.95	0.779	0.19	0.12	1.54	0.412
OPDA	-0.70	0.19	-3.67	**0.001**	-1.43	0.20	-7.03	**<0.001**	-0.39	0.21	-1.82	0.264	-0.74	0.19	-3.99	**<0.001**	0.31	0.19	1.62	0.367	1.05	0.21	5.07	**<0.001**
OH-JA	0.49	0.08	6.29	**<0.001**	0.47	0.08	5.64	**<0.001**	0.51	0.09	5.80	**<0.001**	-0.03	0.07	-0.39	0.980	0.02	0.08	0.23	0.996	0.05	0.09	0.57	0.943
OH-JA-Ile	-0.02	0.13	-0.17	0.998	0.03	0.14	0.24	0.995	0.04	0.14	0.29	0.991	0.05	0.13	0.43	0.973	0.06	0.13	0.49	0.961	0.01	0.14	0.06	1.000
PR1	0.98	0.37	2.68	**0.037**	1.57	0.40	3.90	**<0.001**	-0.00	0.40	-0.00	1.000	0.59	0.35	1.68	0.335	-0.98	0.35	-2.78	**0.028**	-1.57	0.39	-4.01	**<0.001**
Total GLS	15.24	4.07	3.74	**0.001**	27.26	4.37	6.23	**<0.001**	17.67	4.54	3.89	**<0.001**	12.03	3.96	3.04	**0.015**	2.43	4.14	0.59	0.936	-9.59	4.43	-2.16	0.138
sinigrin	14.99	3.99	3.76	**0.001**	26.53	4.28	6.19	**<0.001**	17.38	4.44	3.91	**<0.001**	11.55	3.88	2.98	**0.017**	2.39	4.05	0.59	0.935	-9.15	4.34	-2.11	0.155
4OHI3M	0.06	0.10	0.60	0.932	0.417	0.11	3.96	**<0.001**	0.03	0.11	0.30	0.990	0.36	0.10	3.76	**0.001**	-0.03	0.10	-0.26	0.994	-0.38	0.11	-3.60	**0.002**
2PE	0.02	0.11	0.15	0.999	0.37	0.12	3.20	**0.007**	0.29	0.12	2.49	*0.062*	0.35	0.11	3.39	**0.004**	0.27	0.11	2.60	**0.047**	-0.08	0.12	-0.72	0.890
I3M	0.28	0.12	2.27	0.283	0.95	0.13	7.24	**<0.001**	0.21	0.14	1.53	0.758	0.68	0.12	5.69	**<0.001**	-0.07	0.13	-0.52	0.999	-0.74	0.14	-5.47	**<0.001**

Phytochemistry flowers timepoint 6																								
LOX2	0.00	0.12	0.02	1.000	-0.30	0.13	-2.39	*0.079*	-0.11	0.13	-0.84	0.835	-0.30	0.11	-2.67	**0.038**	-0.11	0.12	-0.95	0.779	0.19	0.12	1.54	0.412
OPDA	-0.70	0.19	-3.67	**0.001**	-1.43	0.20	-7.03	**<0.001**	-0.39	0.21	-1.82	0.264	-0.74	0.19	-3.99	**<0.001**	0.31	0.19	1.62	0.367	1.05	0.21	5.07	**<0.001**
OH-JA	0.49	0.08	6.29	**<0.001**	0.47	0.08	5.64	**<0.001**	0.51	0.09	5.80	**<0.001**	-0.03	0.07	-0.39	0.980	0.02	0.08	0.23	0.996	0.05	0.09	0.57	0.943
OH-JA-Ile	0.47	0.13	3.72	**0.001**	0.42	0.13	3.16	**0.009**	0.43	0.15	2.93	**0.018**	-0.05	0.12	-0.42	0.975	-0.04	0.14	-0.29	0.991	0.01	0.14	0.08	1.000
PR1	0.50	0.36	1.41	0.493	1.42	0.38	3.70	**0.001**	-0.09	0.39	-0.23	0.996	0.91	0.35	2.63	**0.043**	-0.60	0.36	-1.67	0.342	-1.51	0.38	-3.94	**<0.001**
Total GLS	15.24	4.07	3.74	**0.001**	27.26	4.37	6.23	**<0.001**	17.67	4.54	3.89	**<0.001**	12.03	3.96	3.04	**0.015**	2.43	4.14	0.59	0.936	-9.59	4.43	-2.16	0.138
sinigrin	14.99	3.99	3.76	**0.001**	26.53	4.28	6.19	**<0.001**	17.38	4.44	3.91	**<0.001**	11.55	3.88	2.98	**0.017**	2.39	4.05	0.59	0.935	-9.15	4.34	-2.11	0.155
4OHI3M	0.06	0.10	0.60	0.932	0.417	0.11	3.96	**<0.001**	0.03	0.11	0.30	0.990	0.36	0.10	3.76	**0.001**	-0.03	0.10	-0.26	0.994	-0.38	0.11	-3.60	**0.002**
2PE	0.41	0.11	3.91	**<0.001**	0.40	0.11	3.60	**0.002**	0.31	0.12	2.58	**0.049**	-0.01	0.10	-0.13	0.999	-0.11	0.11	-0.97	0.767	-0.09	0.11	-0.81	0.850
I3M	0.28	0.12	2.27	0.283	0.95	0.13	7.24	**<0.001**	0.21	0.14	1.53	0.758	0.68	0.12	5.69	**<0.001**	-0.07	0.13	-0.52	0.999	-0.74	0.14	-5.47	**<0.001**

Table S3. Output of post-hoc analyses for models of preferences of winged *Brevicoryne brassicae* aphids for plant tissues (vegetative or inflorescence tissues) and organs of damaged and undamaged *Brassica nigra* plant pairs. Plants were damaged for 7 days by one of three herbivores: *Athalia rosae*, *Lipaphis erysimi*, or *Delia radicum*, and paired with an undamaged plant. E = estimate, SE = standard error. Bold values indicate results where $P \leq 0.05$.

	Athalia rosae-damaged plant pairs				*Lipaphis erysimi*-damaged plant pairs				*Delia radicum*-damaged plants pairs			
Plant tissue comparisons	E	SE	z	*P*	E	SE	z	*P*	E	SE	z	*P*
Vegetative-Inflorescence	-0.79	0.24	-3.32	**<0.001**	-0.49	0.25	-1.99	**0.046**	-0.68	0.23	-2.92	**0.004**
Plant organ comparisons												
Buds-Bracts	3.11	0.42	7.44	**<0.001**	3.16	0.51	6.18	**<0.001**	3.71	0.58	6.36	**<0.001**
Floral stems-Bracts	0.51	0.52	0.99	0.971	1.01	0.58	1.73	0.628	1.47	0.64	2.29	0.265
Flowers-Bracts	1.73	0.44	3.92	**0.002**	2.55	0.52	4.90	**<0.001**	2.62	0.60	4.37	**<0.001**
Old leaves-Bracts	0.98	0.48	2.05	0.411	2.08	0.53	3.92	**0.002**	1.90	0.62	3.06	**0.038**
Siliques-Bracts	0.51	0.52	0.99	0.971	0.21	0.67	0.33	1.000	1.20	0.66	1.83	0.559
Stems-Bracts	-0.41	0.65	-0.63	0.998	0.81	0.60	1.35	0.860	0.69	0.71	0.98	0.972
Young leaves-Bracts	1.61	0.45	3.60	**0.006**	1.45	0.56	2.60	0.133	2.30	0.61	3.80	**0.003**
Floral stems-Buds	-2.60	0.33	-7.92	**<0.001**	-2.15	0.32	-6.73	**<0.001**	-2.25	0.29	-7.71	**<0.001**
Flowers-Buds	-1.37	0.19	-7.14	**<0.001**	-0.61	0.17	-3.52	**0.009**	-1.01	0.18	-6.09	**<0.001**
Old leaves-Buds	-2.13	0.26	-8.03	**<0.001**	-1.08	0.20	-5.27	**<0.001**	-1.82	0.24	-7.53	**<0.001**
Siliques-Buds	-2.60	0.33	-7.92	**<0.001**	-2.93	0.46	-6.39	**<0.001**	-2.51	0.33	-7.63	**<0.001**
Stem-Buds	-3.51	0.51	-6.92	**<0.001**	-2.35	0.35	-6.72	**<0.001**	-3.02	0.42	-7.22	**<0.001**
Young leaves-Buds	-1.50	0.20	-7.41	**<0.001**	-1.71	0.26	-6.49	**<0.001**	-1.41	0.20	-6.93	**<0.001**
Flower-Floral stems	1.22	0.36	3.40	**0.013**	1.53	0.33	4.61	**<0.001**	1.15	0.32	3.61	**0.006**

Old leaves-Floral stems	0.47	0.40	1.17	0.930	1.07	0.35	3.06	**0.039**	0.43	0.36	1.21	0.916
Siliques-Floral stems	0.00	0.48	0.00	1.000	-0.79	0.54	-1.46	0.802	-0.26	0.42	-0.62	0.998
Stem-Floral stems	-0.92	0.59	-1.55	0.751	-0.20	0.45	-0.45	1.000	-0.77	0.49	-1.57	0.738
Young leaves-Floral stems	1.10	0.37	3.01	**0.046**	0.44	0.39	1.13	0.942	0.84	0.33	2.52	0.163
Old leaves-Flowers	-0.75	0.30	-2.49	0.177	-0.47	0.23	-2.07	0.397	-0.72	0.27	-2.63	0.123
Siliques-Flowers	-1.22	0.36	-3.40	**0.013**	-2.32	0.47	-4.96	**<0.001**	-1.41	0.35	-4.00	**0.002**
Stem-Flowers	-2.14	0.53	-4.05	**0.001**	-1.73	0.36	-4.80	**<0.001**	-1.92	0.44	-4.40	**<0.001**
Young leaves-Flowers	-0.13	0.25	-0.50	1.000	-1.10	0.28	-3.92	**0.002**	-0.31	0.24	-1.30	0.881
Siliques-Old leaves	-0.47	0.40	-1.17	0.930	-1.86	0.48	-3.86	**0.002**	-0.69	0.39	-1.79	0.587
Stem-Old leaves	-1.39	0.56	-2.48	0.180	-1.27	0.38	-3.36	**0.014**	-1.20	0.47	-2.59	0.138
Young leaves-Old leaves	0.63	0.31	2.03	0.422	-0.63	0.30	-2.11	0.371	0.41	0.29	1.40	0.831
Stem-Siliques	-0.92	0.59	-1.55	0.751	0.59	0.56	1.05	0.959	-0.51	0.52	-0.99	0.971
Young leaves-Siliques	1.10	0.37	3.01	**0.045**	1.22	0.51	2.41	0.209	1.10	0.365	3.01	**0.044**
Young leaves-Stem	2.01	0.53	3.79	**0.003**	0.64	0.41	1.54	0.754	1.61	0.45	3.60	**0.006**

Table S4. Output of (generalized) linear models showing the effects of different fixed (herbivore treatment, plant tissue, plant organ) factors on plant preferences of winged *Brevicoryne brassicae* aphids for damaged and undamaged *Brassica nigra* plants. Plants were damaged for 7 days by one of three herbivores: *Athalia rosae*, *Lipaphis erysimi*, or *Delia radicum*. Plant tissues and organs included vegetative tissues; young leaves, old leaves, stems, and inflorescence tissues; developing pods, buds, flowers, bracts, and floral stems. Plant tissue and organ were analysed in separate analyses. Bold values indicate results where $P \leq 0.05$. Italic values indicate results where $P \leq 0.1$.

	Herbivore treatment (H)			Plant tissue (T)			H*T			H			Plant organ (O)			H*O		
	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	P	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*
Athalia rosae	1	3.76	*0.053*	1	10.44	**0.001**	1	0.08	0.782	1	12.05	**<0.001**	7	302.05	**<0.001**	7	7.44	0.384
Lipaphis erysimi	1	0.89	0.345	1	3.84	**0.050**	1	0.64	0.423	1	2.81	*0.094*	7	208.64	**<0.001**	7	5.79	0.565
Delia radicum	1	1.26	0.262	1	8.19	**0.004**	1	0.06	0.811	1	3.67	*0.055*	7	267.51	**<0.001**	7	8.80	0.267

Table S5. Output of statistical models showing the effects of different fixed (herbivore treatment, plant tissue, time point) factors on the relative expression and concentrations of various genes and compounds involved in phytohormone signalling in floral tissues of damaged and undamaged *Brassica nigra* plants. Floral tissues sampled were buds and flowers. Plants were damaged for 1 or 6 days by one of three herbivores: *Athalia rosae*, *Lipaphis erysimi*, or *Delia radicum*. Full profile includes all genes and compounds and was analysed with permutational multivariate analyses of variance (PERMANOVAs). Genes and compounds were analysed with (generalized) linear models (GLMs). Gene expression was expressed as calibrated normalized relative quantity (CNRQ); relative gene expression calculated by correcting the measured gene expression of the target genes with the expression values of the reference genes and inter-run calibrators. Phytohormone concentrations were expressed as ng/g of dry plant biomass. Bold values indicate results where $P \leq 0.05$. Italic values indicate results where $P \leq 0.1$. *LOX2: LIPOXYGENASE 2*, OPDA: 12-oxo-phytodienoic acid, JA: jasmonic acid, JA-Ile: jasmonoyl-L-isoleucine, OH-JA: hydroxy-JA, OH-JA-Ile: hydroxy-JA-Ile, COOH-JA-Ile: carboxy-JA-Ile, SA: salicylic acid, *PR1: PATHOGENESIS-RELATED PROTEIN 1*, ABA: abscisic acid.

	Herbivore treatment (H)			Plant tissue (TI)			Time point (TP)			H*TI			H*TP			TI*TP			H*TI*TP		
	df	R^2/χ^2	*P*	df	R^2/χ^2	*P*	df	R^2/χ^2	*P*	df	R^2/χ^2	*P*	df	R^2/χ^2	*P*	df	R^2/χ^2	*P*	df	R^2/χ^2	*P*
Full profile	3	5.08	**<0.001**	1	59.95	**<0.001**	1	1.63	**<0.001**	3	0	1.000	3	1.46	**0.013**	1	1.83	**<0.001**	3	0.42	0.606
LOX2	3	6.49	*0.090*	1	37.30	**<0.001**	1	0.22	0.635	3	2.41	0.492	3	1.99	0.575	1	6.65	**0.010**	3	3.21	0.360
OPDA	3	76.03	**<0.001**	1	10.23	**0.001**	1	0.18	0.673	3	4.12	0.249	3	4.16	0.245	1	1.51	0.219	3	1.08	0.782
JA	3	0.02	0.999	1	686.30	**<0.001**	1	0.51	0.474	3	1.40	0.707	3	4.39	0.223	1	9.80	**0.002**	3	1.88	0.598
JA-Ile	3	0.05	0.997	1	508.50	**<0.001**	1	1.41	0.236	3	0.535	0.911	3	4.73	0.193	1	9.64	**0.002**	3	3.65	0.301
OH-JA	3	12.66	**0.005**	1	87.26	**<0.001**	1	4.72	**0.030**	3	2.70	0.441	3	25.18	**<0.001**	1	35.95	**<0.001**	3	1.81	0.613
OH-JA-Ile	3	80.29	**<0.001**	1	3.65	*0.056*	1	33.18	**<0.001**	3	46.95	**<0.001**	3	46.73	**<0.001**	1	70.94	**<0.001**	3	36.61	**<0.001**
COOH-JA-Ile	3	2.81	0.422	1	1.03	0.311	1	11.61	**<0.001**	3	0.17	0.983	3	7.05	*0.070*	1	18.28	**<0.001**	3	1.32	0.725
SA	3	2.49	0.477	1	140.50	**<0.001**	1	1.90	0.168	3	3.90	0.273	3	2.85	0.415	1	9.56	**0.002**	3	3.17	0.366
PR1	3	50.66	**<0.001**	1	37.09	**<0.001**	1	0.80	0.370	3	11.91	**0.008**	3	11.27	**0.010**	1	0.07	0.781	3	8.23	**0.041**
ABA	3	2.74	0.433	1	22.43	**<0.001**	1	9.87	**0.002**	3	0.50	0.920	3	2.47	0.480	1	5.99	**0.014**	3	4.40	0.221

Table S6. Output of post-hoc analyses for models of phytochemistry of buds and flowers of damaged and undamaged *Brassica nigra* plants. Plants were damaged for 7 days by one of three herbivores: *Athalia rosae*, *Lipaphis erysimi*, or *Delia radicum*. E = estimate, SE = standard error, *LOX2* = *LIPOXYGENASE 2*, OPDA = 12-oxo-phytodienoic acid, JA = jasmonic acid, JA-Ile = jasmonoyl-L-isoleucine, OH-JA = hydroxy-JA, OH-JA-Ile = hydroxy-JA-Ile, SA = salicylic acid, *PR1* = *PATHOGENESIS-RELATED PROTEIN 1*, ABA = abscisic acid, GLS = glucosinolates, 2PE = gluconasturtiin, I3M = glucobrassicin. Bold values indicate results where $P \leq 0.05$. Italic values indicate results where $P \leq 0.1$.

	Buds VS Flowers			
	E	SE	z	*P*
LOX2	0.51	0.08	6.10	**<0.001**
OPDA	0.34	0.14	2.45	**0.014**
JA	4.61	0.05	85.69	**<0.001**
JA-Ile	2.33	0.04	52.86	**<0.001**
OH-JA	-0.50	0.04	-12.40	**<0.001**
OH-JA-Ile	-0.26	0.05	-5.60	**<0.001**
SA	-0.84	0.06	-13.31	**<0.001**
PR1	-1.19	0.18	-6.78	**<0.001**
ABA	-0.13	0.03	-4.73	**<0.001**
Total GLS	-7.50	2.96	-2.53	**0.012**
Sinigrin	-7.33	2.90	-2.53	**0.012**
2PE	-0.39	0.06	-6.10	**<0.001**
I3M	-0.11	0.05	-1.94	*0.053*

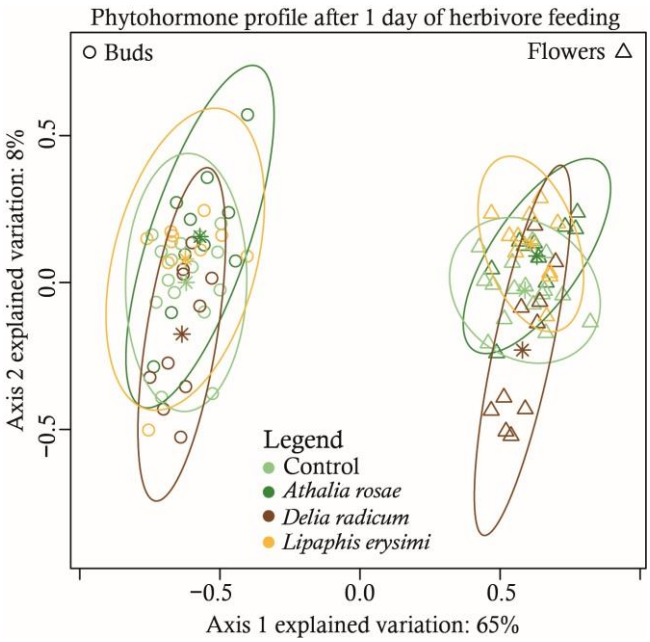

Figure S4. Project to latent structures discriminant (PLS-DA) analysis of phytohormone profiles of floral tissues of damaged and undamaged *Brassica nigra* plants. Profiles were measured in buds (circles) and flowers (triangles) after 1 day of herbivory by larvae of the sawfly *Athalia rosae*, *Lipaphis erysimi* aphids, or larvae of the cabbage root fly *Delia radicum*. Profiles include the upstream jasmonic acid (JA) - marker gene *LIPOXYGENASE 2* (*LOX2*), JA-precursor 12-oxo-phytodienoic acid (OPDA), JA and jasmonoyl-L-isoleucine (JA-Ile), and JA-catabolites hydroxy-JA (OH-JA), hydroxy-JA-Ile (OH-JA-Ile), and carboxy-JA-Ile (COOH-JA-Ile), salicylic acid (SA), downstream SA-marker gene *PATHOGENESIS-RELATED PROTEIN 1* (*PR1*), and abscisic acid (ABA). Gene expression was expressed as calibrated normalized relative quantity (CNRQ); relative gene expression calculated by correcting the measured gene expression of the target genes with the expression values of the reference genes and inter-run calibrators. Phytohormone concentrations were expressed as ng/g of dry plant biomass. Asterisks denote centroids. Highlighted areas denote 95% confidence intervals. Number of replicates per herbivore treatment varied between 8-16.

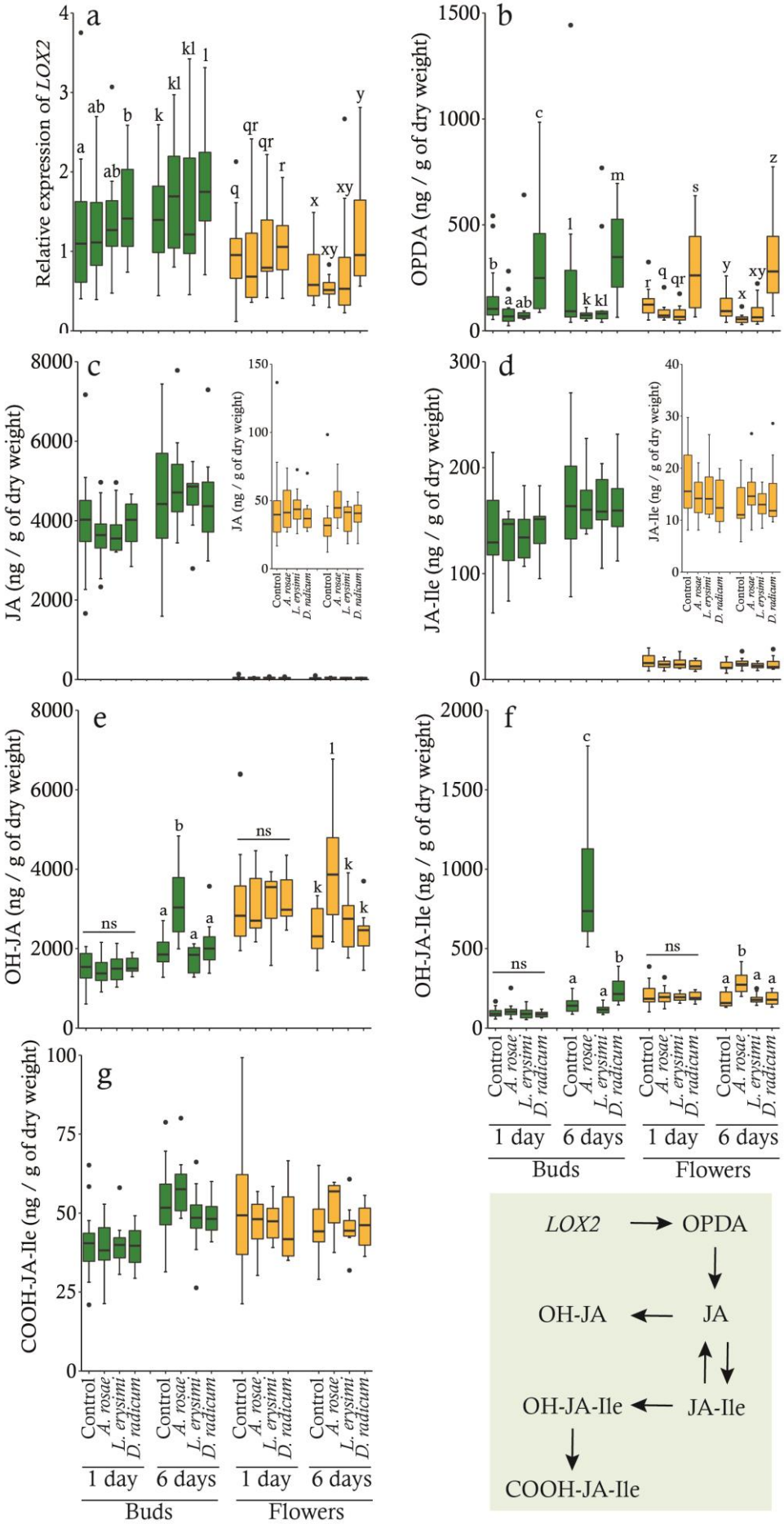
a
Relative expression of LOX2
b
OPDA (ng / g of dry weight)
c
JA (ng / g of dry weight)
d
JA-Ile (ng / g of dry weight)
e
OH-JA (ng / g of dry weight)
f
OH-JA-Ile (ng / g of dry weight)
g
COOH-JA-Ile (ng / g of dry weight)
Control
A. rosae
L. erysimi
D. radicum
1 day
6 days
Buds
Flowers
LOX2
OPDA
JA
OH-JA
JA-Ile
OH-JA-Ile
COOH-JA-Ile

Figure S5. Relative expression and concentrations of various genes and compounds involved in jasmonic acid (JA) pathway signalling in floral tissues of damaged and undamaged *Brassica nigra* plants. Gene expression and concentrations were measured in buds (green) and flowers (yellow) after 1 and 6 days of herbivory by larvae of the sawfly *Athalia rosae*, *Lipaphis erysimi* aphids, or larvae of the cabbage root fly *Delia radicum* as well as for uninfested control plants. Boxplots show median (line), 1st and 3rd quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the 1st or above the 3rd quartile) are represented by circles. Inset panels in c and d are to represent the boxplots below, otherwise not distinguishable. Gene expression was expressed as calibrated normalized relative quantity (CNRQ); relative gene expression calculated by correcting the measured gene expression of the target genes with the expression values of the reference genes and inter-run calibrators. Number of replicates per herbivore treatment varied between 8-16. Number of replicates per herbivore treatment varied between 8-16. Letters above bars indicate significant differences at P ≤ 0.05 based on Tukey's post-hoc tests. Letter groups (a-b, k-l, q-r, x-y) indicate separate comparisons of herbivore treatments within tissue and time point, whereas ns indicates no differences. *LOX2: LIPOXYGENASE 2*, OPDA: 12-oxo-phytodienoic acid, JA-Ile: jasmonoyl-L-isoleucine, OH-JA: hydroxy-JA, OH-JA-Ile: hydroxy-JA-Ile, COOH-JA-Ile: carboxy-JA-Ile.

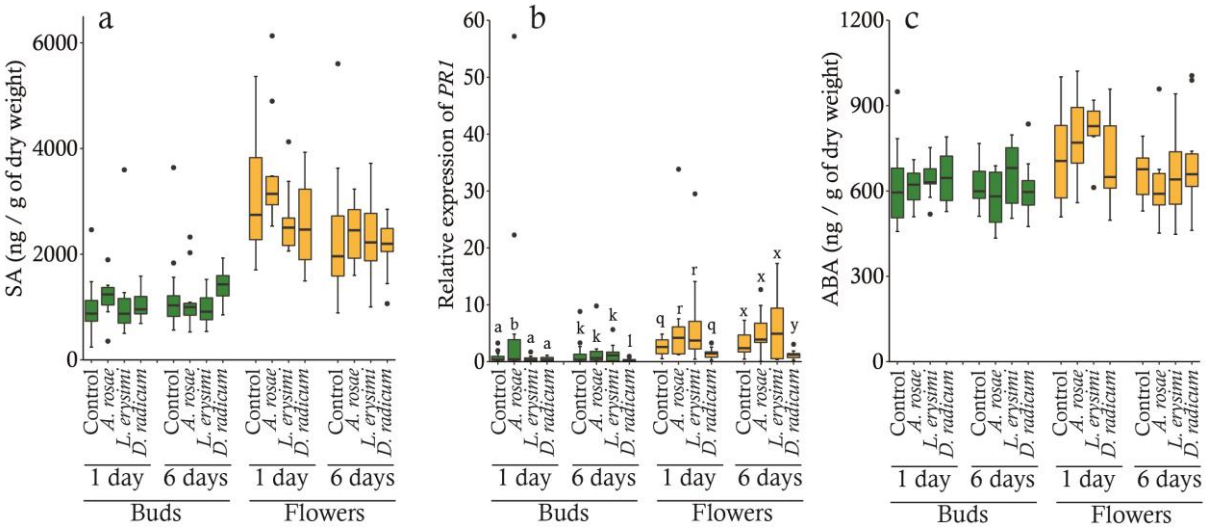

Figure S6. Concentrations of salicylic acid (SA) (a), relative expression of *PATHOGENESIS-RELATED PROTEIN 1* (*PR1*) (b), and concentrations of abscisic acid (ABA) (c) in floral tissues of damaged and undamaged *Brassica nigra* plants. Gene expression and concentrations were measured in buds (green) and flowers (yellow) after 1 and 6 days of herbivory by larvae of the sawfly *Athalia rosae*, *Lipaphis erysimi* aphids, or larvae of the cabbage root fly *Delia radicum*. Boxplots show median (line), 1st and 3rd quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the 1st or above the 3rd quartile) are represented by circles. Gene expression was expressed as calibrated normalized relative quantity (CNRQ); relative gene expression calculated by correcting the measured gene expression of the target genes with the expression values of the reference genes and inter-run calibrators. Number of replicates per herbivore treatment varied between 8-16. Number of replicates per herbivore treatment varied between 8-16. Letter groups (a-b, k-l, q-r, x-y) above bars indicate significant differences with $P \leq 0.05$ between herbivore treatments within tissue and time point based on Tukey's post hoc tests, whereas ns indicates no differences.

Table S7. Output of (generalized) linear models showing the effects of different fixed (herbivore treatment, plant tissue, time point) factors on the concentrations of glucosinolates in floral tissues of damaged and undamaged *Brassica nigra* plants. Floral tissues sampled were buds and flowers. Plants were damaged for 1 or 6 days by one of three herbivores: *Athalia rosae*, *Lipaphis erysimi*, or *Delia radicum*. Glucosinolate concentrations were expressed as µmol/g of dry plant biomass. Bold values indicate results where $P \leq 0.05$. Italic values indicate results where $P \leq 0.1$.

	Herbivore treatment (H)			Plant tissue (TI)			Time point (TP)			H*TI			H*TP			TI*TP			H*TI*TP		
	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*
Total glucosinolates	3	35.67	**<0.001**	1	6.49	**0.011**	1	0.08	0.778	3	1.44	0.696	3	4.67	0.198	1	0.70	0.402	3	0.85	0.838
Sinigrin	3	35.55	**<0.001**	1	6.46	**0.011**	1	0.08	0.783	3	1.42	0.701	3	4.60	0.204	1	0.72	0.395	3	0.90	0.827
4-OH-glucobrassicin	3	19.60	**<0.001**	1	0.39	0.534	1	1.36	0.244	3	2.12	0.548	3	3.57	0.312	1	0.03	0.868	3	0.13	0.989
Glucobrassicin	3	40.18	**<0.001**	1	32.07	**<0.001**	1	0.21	0.650	3	12.42	**0.006**	3	6.21	0.102	1	9.89	**0.002**	3	1.54	0.674
Gluconasturtiin	3	22.35	**<0.001**	1	3.34	*0.067*	1	5.74	**0.017**	3	0.89	0.827	3	11.05	**0.011**	1	0.458	0.499	3	0.11	0.991

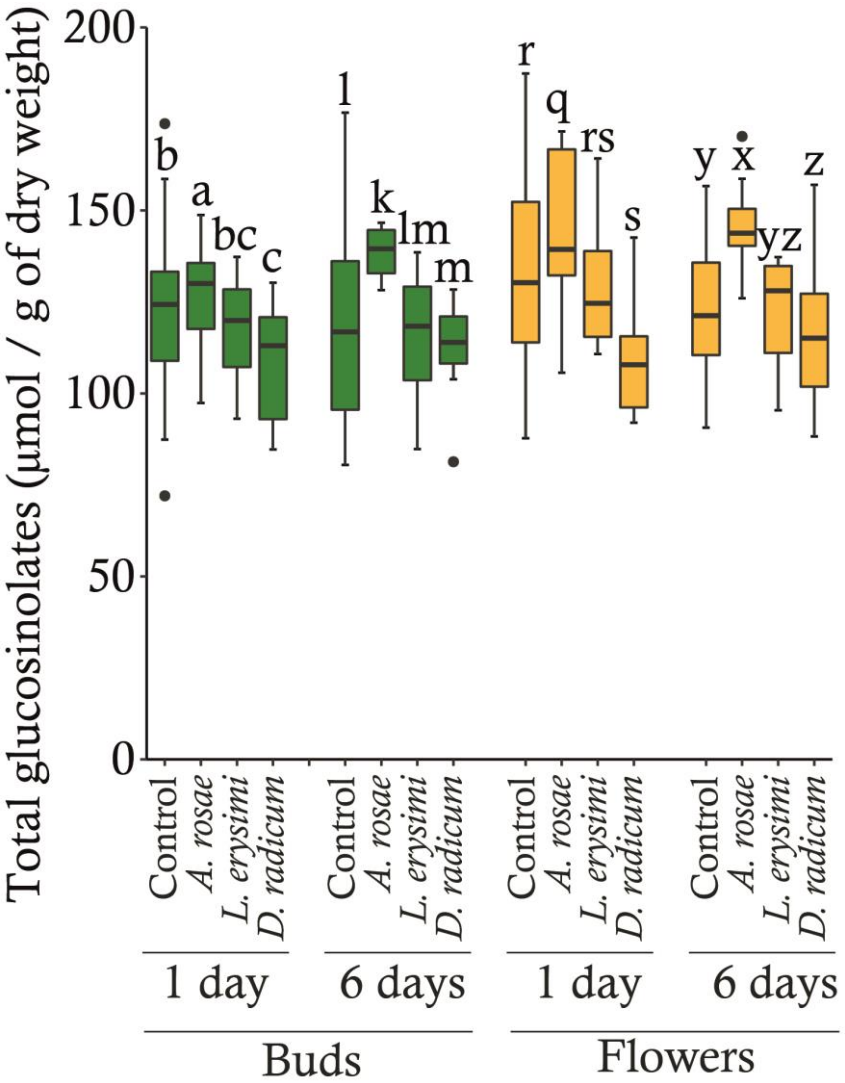

Figure S7. Total glucosinolate concentrations in floral tissues of damaged and undamaged *Brassica nigra* plants. Concentrations were measured in buds (green) and flowers (yellow) after 1 and 6 days of herbivory by larvae of the sawfly *Athalia rosae*, *Lipaphis erysimi* aphids, or larvae of the cabbage root fly *Delia radicum* as well as for uninfested control plants. Boxplots show median (line), 1st and 3rd quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the 1st or above the 3rd quartile) are represented by circles. Number of replicates per herbivore treatment varied between 8-16. Letters above bars indicate significant differences at $P \leq 0.05$ based on Tukey's post-hoc tests. Letter groups (a-b, k-l, q-r, x-y) indicate separate comparisons of herbivore treatments within tissue and time point.

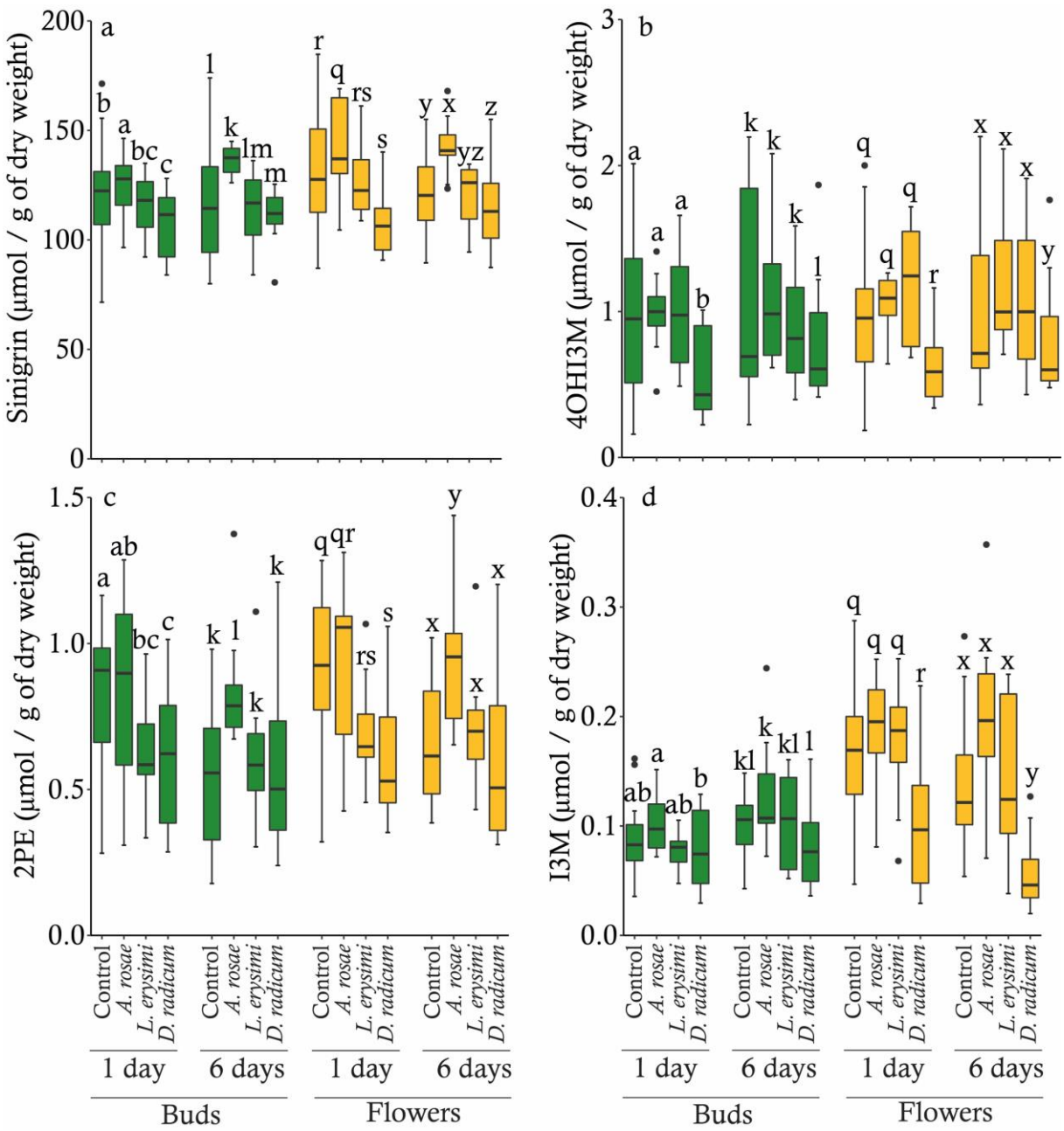

Figure S8. Concentrations of a) sinigrin, b) 4-hydroxy-glucobrassicin (4OHI3M), c) gluconasturtiin (2PE), and d) glucobrassicin (I3M) in floral tissues of damaged and undamaged *Brassica nigra* plants. Concentrations were measured in buds (green) and flowers (yellow) after 1 and 6 days of herbivory by larvae of the sawfly *Athalia rosae*, *Lipaphis erysimi* aphids, or larvae of the cabbage root fly *Delia radicum*. Boxplots show median (line), 1st and 3rd quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the 1st or above the 3rd quartile) are represented by circles. Number of replicates per herbivore treatment varied between 8-16. Letter groups (a-c, k-m, q-s, x-z) above bars indicate significant differences with $P \leq 0.05$ between herbivore treatments within tissue and time point based on Tukey's post hoc tests.