## Supplementary Information

**The selective sequestration of glucosinolates by the cabbage aphid severely impacts a predatory lacewing**

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**Fig. S1. Absolute glucosinolate content in the bodies of adult *B. brassicae* and the host *A. thaliana* wild-type Col-0 plants during a 6-day aphid infestation.**

**Fig. S2. Expression of the *bmy* gene and BMY enzymatic activities are independent of developmental stages and host glucosinolate content.**

**Fig. S3. SDS-Page of the recombinant BMY protein produced in *E. coli* cells**.

**Fig. S4. Profiling metabolites of the indolic glucosinolate I3M-GSL in response to tissue damage.**

**Table S1. Primer sets for gene cloning and qRT-PCR.**

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**Supplementary methods**

**Fig. S1. Absolute glucosinolate contents in the bodies of adult *B. brassicae* aphids and the host *A. thaliana* wild-type Col-0 plants during a 6-day aphid infestation. (a)** 3MSOP-GSL (organisms: *F1,54* = 13.51, *P*≤ 0.001; duration: *F8,54* = 4.700, *P*≤ 0.001; organisms×duration: *F8,54* = 7.193, *P*≤ 0.001; *n*= 4 in all points), **(b)** 4MSOB-GSL (organisms: *F1,54* = 477.8, *P*≤ 0.001; duration: *F8,54* = 224.0, *P*≤ 0.001; organisms×duration: *F8,54* = 264.8, *P*≤ 0.001; *n*= 4 in all points), **(c)** 5MSOP-GSL (organisms: *F1,54* = 222.0, *P*≤ 0.001; duration: *F8,54* = 120.0, *P*≤ 0.001; organisms×duration: *F8,54* = 128.5, *P*≤ 0.001; *n*= 4 in all points), **(d)** 8MSOO-GSL (organisms: *F1,54* = 290.8, *P*≤ 0.001; duration: *F8,54* = 68.18, *P*≤ 0.001; organisms×duration: *F8,54* = 72.23, *P*≤ 0.001; *n*= 4 in all points), **(e)** 4MTB-GSL (organisms: *F1,54* = 57.86, *P*≤ 0.001; duration: *F8,54* = 31.12, *P*≤ 0.001; organisms×duration: *F8,54* = 45.46, *P*≤ 0.001; *n*= 4 in all points), **(f)** I3M-GSL (organisms: *F1,54* = 18.97, *P*≤ 0.001; duration: *F8,54* = 16.17, *P*≤ 0.001; organisms×duration: *F8,54* = 28.71, *P*≤ 0.001; *n*= 4 in all points), **(g)** 1MOI3M-GSL (organisms: *F1,54* = 0.05, *P*= 0.824; duration: *F8,54* = 11.12, *P*≤ 0.001; organisms×duration: *F8,54* = 13.02, *P*≤ 0.001; *n*= 4 in all points), and **(h)** 4MOI3M-GSL (organisms: *F1,54* = 279.7, *P*≤ 0.001; duration: *F8,54* = 42.14, *P*≤ 0.001; organisms×duration: *F8,54* = 7.502, *P*≤ 0.001; *n*= 4 in all points). Significant differences (*P*≤ 0.05) between means (±s.e.) were determined by Tukey HSD tests in conjunction with two-way ANOVA**.**

**Fig. S2. Expression of the *bmy* gene and BMY enzymatic activities are independent of developmental stages and host glucosinolate content. (a** and **b)** qRT-PCRanalysis of *bmy* gene expression in three *B. brassicae* life stages (**a**, *F2,12* = 2.972, *P=* 0.089, *n*= 5 in all bars), and in 4th-instar nymphs fed on three *A. thaliana* genotypes (**b**, *F2,12* = 1.348, *P=* 0.296, *n*= 5 in all bars). Myrosinase enzymatic activities towards two selected glucosinolates using aphid protein extracts either from three life stages **(c** and **e)** or from the 4th-instar nymphs fed on three different host plants **(d** and **f)**. **(c)**, *F2,9* = 1.674, *P=* 0.241, *n*= 4 in all bars; **(d)**, *F2,9* = 0.508 *P=* 0.618, *n*= 4 in all bars; **(e)**, *F2,9* = 0.859, *P=* 0.456, *n*= 4 in all bars; **(f)**, *F2,9* = 0.759, *P=* 0.496, *n*= 4 in all bars. Significant differences (*P*≤ 0.05) between means (± s.e.) were determined by Tukey HSD test in conjunction with one-way ANOVA.



**Fig. S3. SDS-PAGE of the recombinant BMY protein produced in *E. coli* cells**. Molecular weight markers are indicated in kilodalton (kDa).

**Fig. S4. Profiling metabolites of the indolic glucosinolate I3M-GSL in response to tissue damage. (a)** An overview of the pathways of I3M metabolism. **(b)** Reduction of I3M-GSL induced by tissue damage (*F2,12*= 11.06, *P* ≤ 0.01; *n*= 5 in all bars). **(c)** An extracted LC-MS/MS chromatogram shows detectable I3M-GSL-derivatives in *B. brassicae*. Note that this method cannot detect I3M-ITC. **(d)** Indole-3-acetonitrile (I3M-CN) was easily detected by this method (the chromatogram of an authentic standard is shown), but was not detectable in aphid samples. Significant differences (*P*≤ 0.05) between means (± s.e.) were determined by Tukey HSD test in conjunction with a one-way ANOVA in **b**.

**Table S1. Primer sets for gene cloning and qRT-PCR.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Primer (5’🡪3’)** | **Function** | **Gene accession** |
| *bmyFLF* | AATATGGATTATAAATTTCCAAAGG | Recombination BMY in *E. coli* cells | AF203780 |
| *bmyFLR* | TGGTTTGCCAGTTGATACCAC |
| *bmyVF* | GCGGCGGGATCCAATATGGATTATAAATTTCCAAAGG |
| *bmyVR* | GCGGCGCTCGAGTGGTTTGCCAGTTGATACCAC |
| *bmyQF* | AGGCTGGAATGAAGACGGAA | qPCR |
| *bmyQR* | GGAATCACAGGCAATATCTCCA |
| *EF-1αF* | ACCACCATACAGCGAAAGCC | AY219734 |
| *EF-1αR* | GATGGGCACGAAAGCAACTG |

**Table S2. Substrates used for enzyme assays and external standards used for quantification.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compounds** | **Other names** | **Supplier** | **CAS#** | **CAT#** |
| 4MSOB-GSL | Glucoraphanin | Carl Roth, Karlsruhe, Germany | 21414-41-5 | 2374.1 |
| 4MSOB-CN | 4-(Methylsulfinyl)butanenitrile | Synthesized as described in (Beran et al., 2018) | 61121-65-1 |  |
| 4MSOB-ITC | D,L-Sulforaphane | BIOZOL Diagnostica Vertrieb, Eching, Germany | 142825-10-3 | USB-289262 |
| 4MSOB-ITC-GSH | D,L-Sulforaphane glutathione | Santa Cruz Biotechnology, Dallas, TX, United States | 289711-21-3 | sc-207496 |
| 4MSOB-ITC-CG | Sulforaphane-cysteine-glycine | Synthesized as described in (Schramm et al., 2012) |  |  |
| 4MSOB-ITC-Cys | D,L-Sulforaphane-L-cysteine | Santa Cruz Biotechnology, Dallas, TX, United States | 364083-21-6 | sc-207499 |
| 4MSOB-ITC-NAC | D,L-Sulforaphane N-acetyl-L-cysteine | Santa Cruz Biotechnology, Dallas, TX, United States | 334829-66-2 | sc-207497 |
| Allyl glucosinolate | Sinigrin | Carl Roth, Karlsruhe, Germany | 3952-98-5 | 5319.1 |
| 3MSOP-GSL | Glucoiberin | Phytoplan, Heidelberg, Germany | 15592-34-4 | 3413.99 |
| 5MSOP-GSL | Glucoalyssin | Phytoplan, Heidelberg, Germany | 499-37-6 | 3428.97 |
| 8MSOO-GSL | Glucohirsutin | Phytoplan, Heidelberg, Germany | 21973-60-4 | 3438.97 |
| Benzyl-GSL | Glucotropaeolin | Santa Cruz Biotechnology, Dallas, TX, United States | 5115-71-9 | sc-285861 |
| *p*-Hydroxybenzyl glucosinolate | Sinalbin | Carl Roth, Karlsruhe, Germany | 16411-05-5 | 6694.1 |
| I3M-GSL | Glucobrassicin | Carl Roth, Karlsruhe, Germany | 4356-52-9 | 2279.1 |
| 1MOI3M-GSL | Neoglucobrassicin | Phytoplan, Heidelberg, Germany | 5187-84-8 | 3434.97 |
| 4MOI3M-GSL | 4-Methoxyglucobrassicin | Phytoplan, Heidelberg, Germany | 83327-21-3 | 3433.94 |

**Table S3. LC-MS/MS parameters used for multiple reaction monitoring (MRM) analyses.** Q1, *m*/*z* of quadrupole one; Q3, *m*/*z* of quadrupole three; DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential; B, mobile phase B (acetonitrile)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Metabolites** | **Ionization mode and elution profile** | **Q1 *m*/*z*** | **Q3 *m*/*z*** | **Retention time (min)** | **DP (V)** | **EP (V)** | **CE (V)** | **CXP (V)** |
| 3MSOP-GSL | Negative modeThe elution profile was: 0-1 min, 1.5% B; 1-6 min, 1.5-5% B; 6-8 min, 5-7% B; 8-18 min, 7-21% B; 18-23 min, 21-29% B; 23-23.1 min, 29-100% B; 23.1-24min 100% B and 24.1-28 min 1.5% B; at a flow rate of 1.0 mL/min. | 421.80 | 95.90 | 5.80 | -95.00 | -4.50 | -60.00 | 0.00 |
| 4MSOB-GSL | 435.90 | 95.80 | 8.00 | -95.00 | -5.00 | -60.00 | 0.00 |
| 8MSOO-GSL | 492.10 | 95.80 | 19.00 | -105.00 | -4.50 | -58.00 | 0.00 |
| 5MSOP-GSL | 449.90 | 95.80 | 11.60 | -95.00 | -5.00 | -60.00 | 0.00 |
| 7MSOH-GSL | 477.90 | 95.80 | 16.50 | -95.00 | -5.00 | -60.00 | 0.00 |
| 4MTB-GSL | 419.90 | 95.90 | 18.00 | -90.00 | -11.00 | -58.00 | 0.00 |
| I3M-GSL | 447.00 | 95.80 | 20.00 | -95.00 | -12.00 | -50.00 | 0.00 |
| 1MOI3M-GSL | 477.00 | 95.80 | 24.40 | -95.00 | -12.00 | -50.00 | 0.00 |
| 4MOI3M-GSL | 477.10 | 95.80 | 22.00 | -95.00 | -12.00 | -50.00 | 0.00 |
| 4MSOB-CN | Positive modeThe elution profile was: 0-0.5 min, 3-15% B; 0.5-2.5 min, 15-85% B; 2.5-2.6 min, 85-100% B; 2.6-3.5 min 100% B and 3.5-6.0 min 3% B; at a flow rate of 1.1 mL/min. | 146.00 | 129.00 | 1.60 | 63.00 | 10.00 | 13.00 | 4.00 |
| 4MSOB-ITC | 178.11 | 114.00 | 2.50 | 51.00 | 5.00 | 13.00 | 4.00 |
| 4MSOB-ITC-GSH | 485.11 | 179.10 | 2.00 | 76.00 | 5.50 | 29.00 | 6.00 |
| 4MSOB-ITC-CG | 356.07 | 136.10 | 1.90 | 46.00 | 11.00 | 15.00 | 4.00 |
| 4MSOB-ITC-Cys | 299.06 | 136.10 | 1.70 | 51.00 | 3.00 | 15.00 | 4.00 |
| 4MSOB-ITC-Cyc | 265.11 | 201.00 | 1.10 | 51.00 | 8.50 | 25.00 | 4.00 |
| 4MSOB-ITC-NAC | 341.07 | 178.10 | 2.20 | 51.00 | 3.00 | 17.00 | 6.00 |
| I3C | Positive modeThe elution profile was: 0-0.5 min, 10% B; 0.5-6.0 min, 10-90% B; 6.0-6.1 min, 90-100% B; 6.1-7.5 min 100% B and 7.5-10.0 min 10% B; at a flow rate of 1.0 mL/min. | 146.058 | 118.10 | 1.18 | 66.00 | 3.50 | 19.00 | 4.00 |
| I3C-CN (IAN) | 157.168 | 130.00 | 4.51 | 51.00 | 7.50 | 15.00 | 4.00 |
| I3M-Ascorbate | 306.036 | 130.10 | 3.16 | 36.00 | 8.00 | 21.00 | 4.00 |
| I3M-GSH | 437.03 | 308.00 | 2.52 | 41.00 | 3.00 | 19.00 | 12.00 |
| I3M-CG | 308.016 | 76.00 | 2.71 | 106.00 | 4.50 | 35.00 | 4.00 |
| I3M-NAC | 293.029 | 76.90 | 4.60 | 21.00 | 8.00 | 83.00 | 4.00 |

**Supplementary methods**

**RNA isolation, cDNA synthesis and quantitative real-time PCR (qPCR)**

*Brevicoryne brassicae* endogenous myrosinase-encoding gene (*bmy*) transcripts were quantified at different aphid life stages and for different diets (with or without glucosinolates). *B. brassicae* 2nd- and 4th-instar nymphs and adults were fed on *A. thaliana* wild-type plants, and 4th instar nymphs were fed on *A. thaliana* *myb28myb29* and *myb28myb29cyp79b2cyp79b3* plants. Aphids from each treatment were pooled into TRIzol reagent (Invitrogen, Waltham, MA, USA) in 1.5 mL Eppendorf tubes (5 replicates per treatment, each replicate tube containing around 10 aphids) and then kept at 4 °C before use. Total RNA was isolated from stored aphids according to the manufacturer’s protocol and was subjected to DNaseI (Thermo Fisher Scientific, Waltham, MA, USA) treatment to eliminate genomic DNA contamination. cDNA was synthesized from this RNA using a SuperScript III Reverse transcriptase kit (Invitrogen). The numbers of gene transcripts in these cDNA samples were measured using qPCR performed with Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent Technologies, Santa Clara, CA, USA). The elongation factor 1 alpha-encoding (*EF-1α)* gene was used as an internal control to normalize the abundance of genetranscripts. All the gene accession numbers and primer pairs, which were designed via Primer3 software version 4.0, are listed in Table S1.

**Myrosinase activity of *B. brassicae* protein extract**

To determine the myrosinase activity of *B. brassicae* at different life stages and while feeding on different food sources (with or without glucosinolates), protein extracts were prepared from *B. brassicae* 2nd- and 4th-instar nymphs and adults fed with *A. thaliana* wild-type plants, and 4th-instar nymphs fed with *A. thaliana* *myb28myb29* and *myb28myb29cyp79b2cyp79b3* plants. Approximately 6 mg of aphids were pooled into 1.5 mL Eppendorf tubes as one sample (4 replicates per treatment). Samples were homogenized in ice-cold citric acid buffer (200 μL, 50 mM, 10% glycerol; pH 4.1) with ceramic beads using a homogenizer for 3 min. Homogenized samples were centrifuged at 13,000 x *g* for 20 min at 4 °C to separate undissolved particles. Clear supernatants were transferred to 1.5 mL Eppendorf tubes, and 20 μL of each sample were used to measure protein concentration using the Bradford Reagent (Serva Electrophoresis, Heidelberg, Germany) according to the manufacturer’s instructions. A 0.5 µg quantity of extracted protein for each sample was dissolved in citric acid buffer (50 µL, 50 mM, pH 4.1) and reacted with 1 mM glucosinolate at 28 °C for 10 min. An aliquot of 10 µL of the reaction solution was taken out at 0 min and 10 min time points and added to 90 µL of pure methanol to immediately stop the reaction. The concentration of glucosinolates was determined by LC-MS/MS as described below and the amount of glucosinolate reacted was calculated based on the concentration at the 0 min point minus the concentration at the 10 min point. Aliphatic 4MSOB-GSL (4-methylsulfinylbutyl glucosinolate) and indolic I3M-GSL (indolyl-3-methyl glucosinolate) were used for the assays.

**Recombinant BMY expression and purification**

To determine the specific activity of *B. brassicae* endogenous BMY with different glucosinolates, BMY protein was produced in *Escherichia coli* cells. The complete coding sequence of the *bmy* gene was retrieved from (Jones et al., 2002). The full length *bmy* mRNA sequence was cloned from the synthesized cDNA pool obtained from the primer pair *bmy*FLFand *bmy*FLR. XhoI and BamHI restriction enzyme cutting sites were added to the ends of the full-length *bmy* using the primer pairs *bmy*VF and *bmy*VR, and the fragment was further digested by XhoI and BamHI (Thermo Fisher Scientific). The pET28a vector used to express the target gene was also digested by XhoI and BamHI. The restriction enzyme-digested *bmy* was inserted into the XhoI-BamHI-cut pET28a cloning site by T4 DNA ligase (Invitrogen). BL21 (DE3) *E. coli* cells (Invitrogen) were transformed by the vector containing the *bmy* insertion by chemical transfection. Simultaneously, *E. coli* BL21 (DE3) cells containing pET28a empty vector were used as negative control.

The histidine-tagged recombinant BMY was affinity purified over Ni-NTA agarose resin (Qiagen, Hilden, Germany). *E. coli* cells were collected in 50 mL Falcon tubes and centrifuged (13,000 x *g* at 4 °C for 30 min). The supernatant was discarded and the pellet was resuspended in lysis buffer (1.5 mL, 50 mM Tris, 20 mM imidazole, 500 mM NaCl, 10% glycerol, and 0.5% Tween 20; pH 7.5) with protease inhibitor mix HP (1:100 v/v) (Serva Electrophoresis) and Benzonase (2 μL/10 mL lysis buffer) (Merck KGaA, Darmstadt, Germany). The cells were lysed by an ultrasonic homogenizer (Sonoplus HD 2070, Bandelin, Berlin, Germany). The supernatant of the lysed cells was collected after centrifugation (13,000 x *g* at 4 °C for 30 min) and transferred to equilibrated Ni-NTA agarose resin in 2 mL Eppendorf tubes. The binding of the histidine tagged protein to the Ni-NTA agarose resin was accomplished by mixing under circular rotation at 4 °C for 1 hour. The collected Ni-NTA agarose resin was washed twice using wash buffer (50 mM Tris, 20 mM imidazole, 500 mM NaCl, and 10% glycerol; pH 7.5). Then the protein was eluted from Ni-NTA agarose resin using elution buffer (50 mM Tris, 250 mM imidazole, 500 mM NaCl, and 10% glycerol; pH 7.5). The buffer was exchanged to a citric acid buffer (50 mM, and 10% glycerol; pH 4.1) using Amicon Ultra-30 K centrifugal filter units (Merck). The purity of the eluted recombinant proteins was analyzed by SDS‐PAGE (BioRad, Hercules, California, USA).

**Glucosinolate extraction from *A. thaliana* Col-0 wild-type plants**

To measure BMY activity with *A. thaliana* wild-type glucosinolates, aboveground tissues of 3 six week-old *A. thaliana* wild-type plants were harvested and immediately frozen in liquid nitrogen to avoid activation of glucosinolates by plant myrosinases. Subsequently, plants were thoroughly freeze-dried using an Alpha 1-4 LDplus freeze dryer (Martin Christ, Osterode am Harz, Germany) for 2 days and homogenized by shaking with 5-6 metal balls (diameter 3 mm) per tube. The extraction of glucosinolates from the plants was accomplished by incubating with 10 mL of methanol:water (80:20) per gram dry weight and shaking for 5 min. The supernatant collected after centrifugation (4000 x *g* at 4 °C for 30 min) was passed through Amicon Ultra-10 K centrifugal filters (Merck) to retain plant proteins including plant myrosinases. The flow-through was then evaporated to remove the solvent on a rotary evaporator (BÜCHI Rotavapor R-114, Büchi Labortechnik AG, Essen, Germany) and resuspended in 1 mL milliQ water for further use.

**Metabolite extraction**

The weighed *B. brassicae* aphids (approximately 6 mg per sample) and *A. thaliana* wild-type plants (approximately 50 mg per sample) were further homogenized in 200 μL and 500 μL extraction solvent (60% methanol in water pH 3.0), respectively, with ceramic beads (Sigmund Lindner, Warmensteinach, Germany) using a Skandex S-7 homogenizer (Grootec GmbH, Kirchheim, Germany) for 3 min. Homogenized samples were centrifuged at 13,000 x *g* for 20 min at room temperature to separate undissolved particles. Clear supernatants were transferred to 2 mL amber glass vials with 0.3 mL glass inserts and analyzed by LC-MS/MS.

**Fe (II) concentration measurement**

To compare the Fe (II) content of *B. brassicae* aphids with that of their host plants, 4th-instar aphids feeding on *A. thaliana* wild-type or *myb28myb29* plants and samples of the corresponding host plants were collected. Around 10 aphids from each plant were pooled as one sample (6 replicates per treatment). Simultaneously, host plant leaves were collected in 5 mL tubes (6 replicates per treatment). Samples were immediately frozen in liquid nitrogen. Leaf materials were ground using a tissue-grinding pestle. The weighed (FW, fresh weight) *B. brassicae* aphids (approximately 5 mg per sample) and *A. thaliana* plant materials (approximately 50 mg per sample) were further homogenized in 200 μL and 500 μL of 5% TCA solution, respectively, with ceramic beads using a homogenizer for 3 min. Homogenized samples were centrifuged at 13,000 x *g* for 20 min at room temperature to separate undissolved particles. A 40 μL aliquot of the clear supernatant from each sample was used for a Fe (II) assay using a ferrozine chromogenic method with an iron assay kit (Japan Institute for the Control of Aging, Shizuoka, Japan) according to the manufacturer's instructions. The absorbance of colored products was measured at 560 nm using an Infinite M200 Luminescence Microplate Reader (Tecan).

**LC-MS/MS analyses**

Intact glucosinolates and metabolites of 4MSOB-GSL and I3M-GSL were detected on an Agilent Technologies 1200 Series HPLC (Agilent Technologies) coupled to an API 5000 triple-quadrupole mass spectrometer (Applied Biosystems Sciex, Darmstadt, Germany). Intact glucosinolates were analyzed by loading samples on to a Nucleodur Sphinx RP column (250 × 4.6 mm × 5 μm, Macherey-Nagel, Düren, Germany) with mobile phase A (0.2% formic acid in milliQ water) and mobile phase B (acetonitrile). 4MSOB-CN, 4MSOB–ITC and its conjugates were analyzed by loading samples onto an Agilent Zorbax Eclipse XDB-C18 column (50 × 4.6 mm × 1.8 μm, Agilent Technologies, Waldbronn, Germany) with mobile phase A (0.05% formic acid in milliQ water) and mobile phase B (acetonitrile). I3C-CN, I3C and its conjugates were analyzed by loading samples onto the same column with mobile phase A (10 mM ammonium formate in milliQ water) and mobile phase B (acetonitrile). Quantification of each compound was achieved by multiple reaction monitoring (MRM) of specific parent to product ion conversions for each compound using external calibration curves; the origin of the external standards is listed in Table S2.. Detailed parameters and the elution profiles are described in Table S3. Analyst 1.5 software (Applied Biosystems) was used for data acquisition and processing.

**References**

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