MOLECULAR ECOLOGY

Supplemental Information for:

Detoxification of plant defensive glucosinolates by an herbivorous caterpillar is beneficial to its endoparasitic wasp

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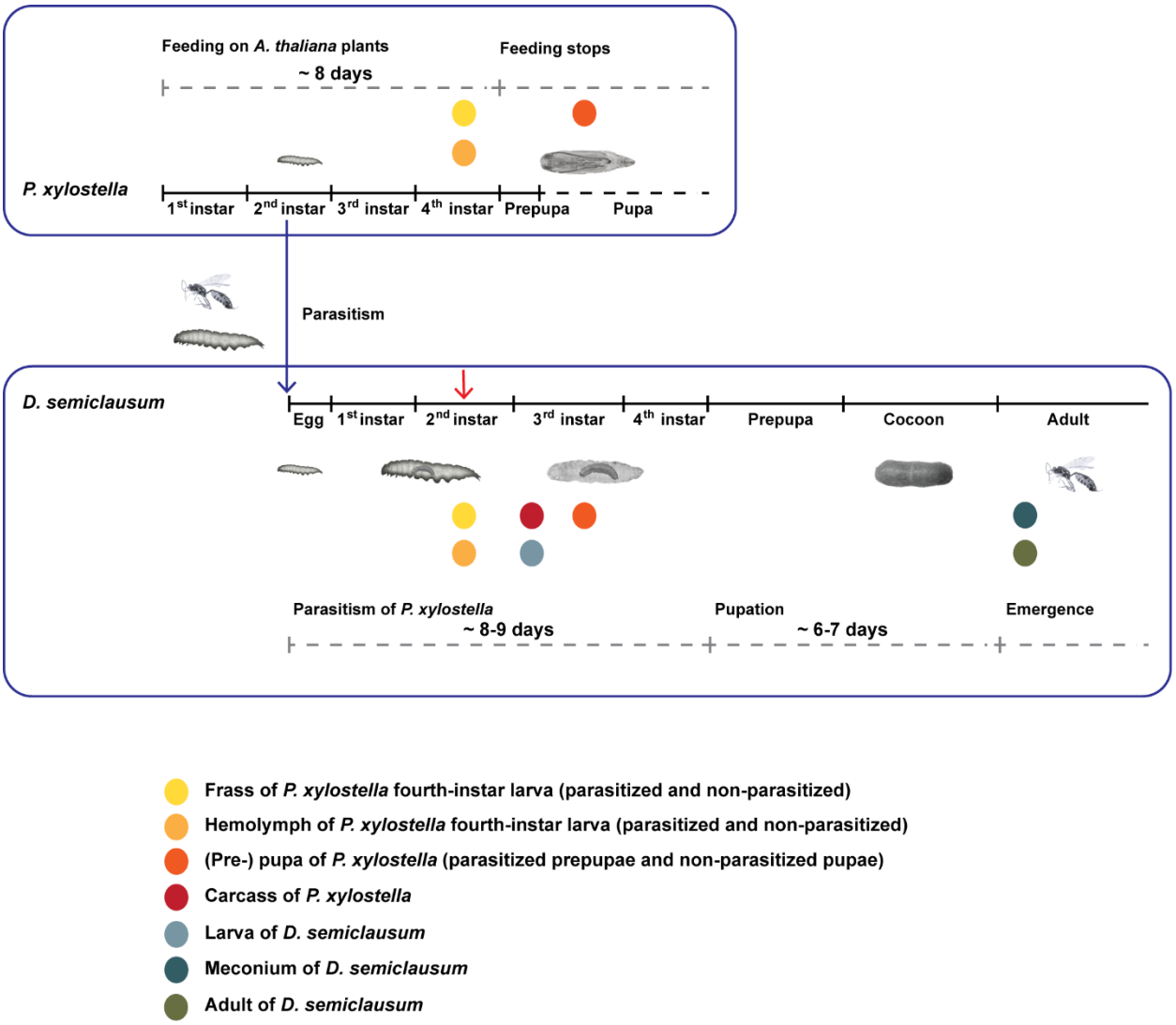

Figure S1 Experimental time course for *Diadegma semiclausum* parasitism of *Plutella xylostella* and sampling points for metabolic analyses. Non-silenced and *Pxgss*-silenced *P. xylostella* second-instar larvae were exposed to adult female *D. semiclausum* for parasitism, and then fed on either *A. thaliana* wild type Col-0 or *myb28myb29* plants until feeding stopped. Hemolymph and frass of fourth-instar non-parasitized and parasitized *P. xylostella* larvae, prepupae of parasitized *P. xylostella*, pupae of non-parasitized *P. xylostella*, third-instar *D. semiclausum* larva and corresponding *P. xylostella* carcass, meconium excreted by *D. semiclausum* when pupating, and adults of *D. semiclausum* were collected for

analyses by LC-MS/MS. Parasitized *P. xylostella* fourth-instar larvae (time point is marked by red arrow in the graph) were collected for gene expression analyses by qRT-PCR.

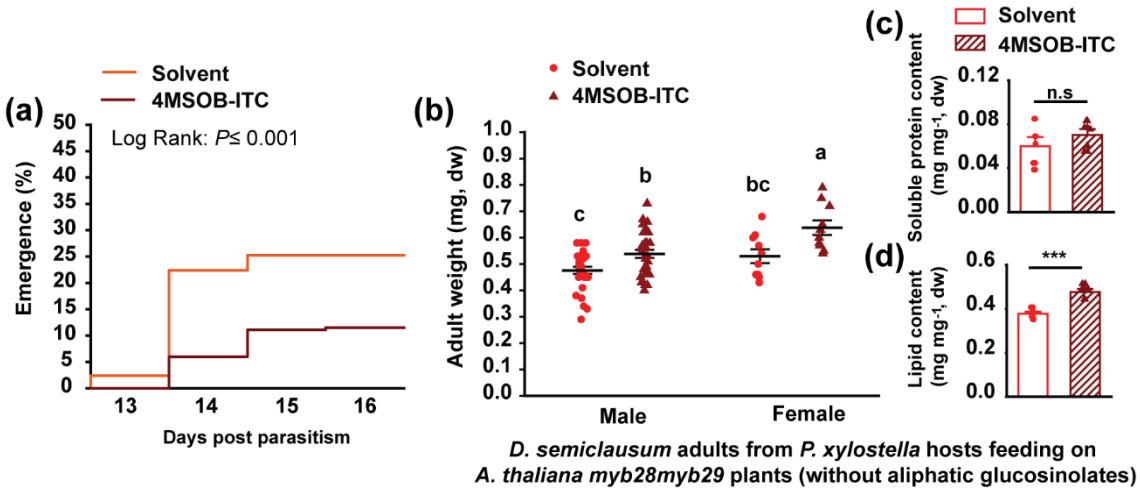

Figure S2 Exposure of *P. xylostella* larvae to 4MSOB-ITC causes the same effects on *D. semiclausum* as *Pxgss* silencing. *D. semiclausum*-parasitized *P. xylostella* larvae were fed on *A. thaliana myb28myb29* leaves infiltrated with either 4MSOB-ITC (in a solution of 0.4% aqueous ethanol) or solvent only as a control. The following variables were measured: **(a)** adult emergence percentage (Log Rank, *df*= 1, *P*≤ 0.001; *n*= 210 and 217, respectively for solvent and 4MSOB-ITC treatments), **(b)** adult dry weight (sex, $F_{1,76}$= 13.475, *P*≤ 0.001; treatment, $F_{1,76}$= 16.778, *P*≤ 0.001; sex×treatment, $F_{1,76}$= 1.156, *P*= 0.286; male, *n*= 30 in all treatments; female, *n*= 10 in all treatments), **(c)** soluble protein content (*t*= 1.044, *P*= 0.332, *n*= 5 in all bars) and **(d)** lipid content (*t*= 5.713, *P*≤ 0.001, *n*= 5 in all bars) in *D. semiclausum* male adults. Significant differences (*P*≤ 0.05) were determined by Kaplan-Meier survival analysis tests in **a**, and significant differences (*P*≤ 0.05) between means (± s.e.) were determined by Tukey HSD tests in conjunction with two-way ANOVA in **b**, and two-tailed *t*-tests for two independent means in **c,d**.

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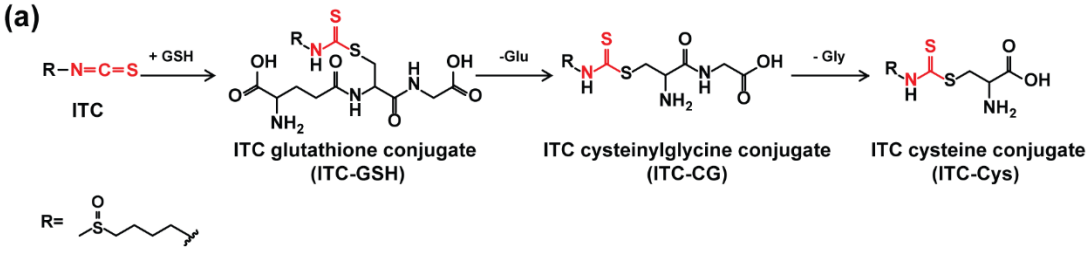

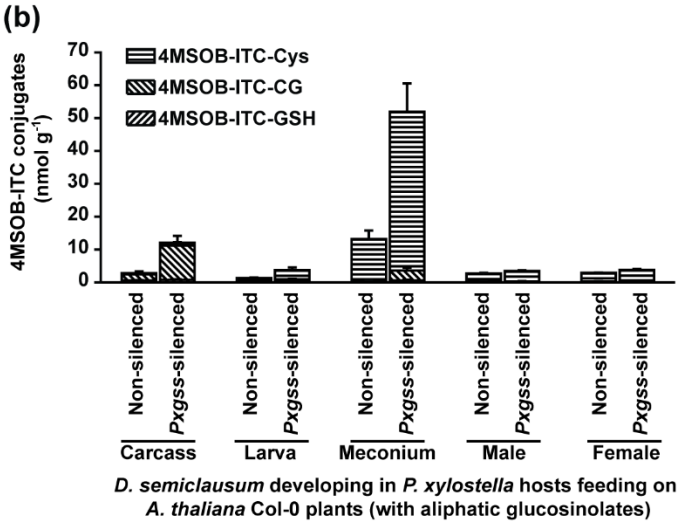

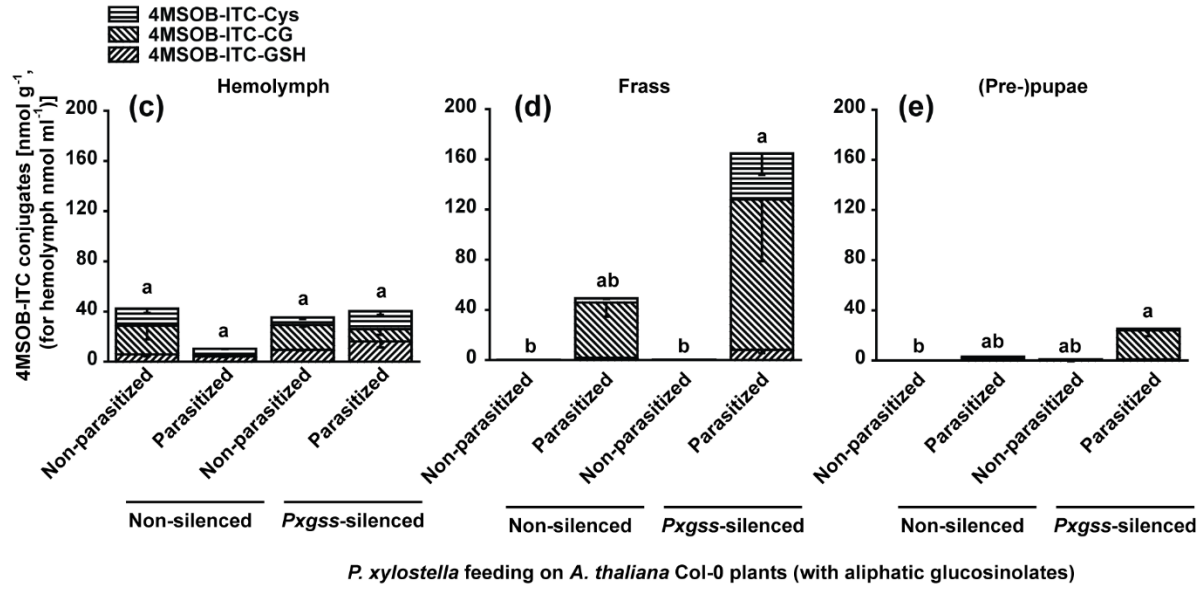

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Figure S3 4MSOB-ITC and its mercapturic acid pathway conjugates are present in non-parasitized and parasitized *P. xylostella* (*Pxgss*-silenced or non-silenced) fed with *A. thaliana* Col-0 plants (containing aliphatic glucosinolates). (a) General mercapturic acid pathway used for detoxification of 4MSOB-ITC in various insects. Ingested 4MSOB-ITC is detoxified by conjugation with glutathione (4MSOB-ITC-GSH), followed by hydrolytic cleavages to form the 4MSOB-ITC-cysteinylglycine (4MSOB-ITC-CG) and 4MSOB-ITC-cysteine conjugates (4MSOB-ITC-Cys). **(b)** 4MSOB-ITC conjugates were quantified in the carcass of *P. xylostella* prepupae, third-instar larvae of *D. semiclausum*, meconium left in the cocoon and adults of *D. semiclausum*, in which *D. semiclausum* parasitized either non-silenced or *Pxgss*-silenced *P. xylostella*. 4MSOB-ITC conjugates were present in **(c)** hemolymph (*gss*-silencing, $F_{1,16}$= 1.553, P= 0.231; parasitism, $F_{1,16}$= 2.164, P= 0.161; *gss*-silencing×parasitism, $F_{1,16}$= 4.009, P= 0.062; n= 5 in all bars) and **(d)** frass (*gss*-silencing, $F_{1,16}$= 2.758, P= 0.116; parasitism, $F_{1,16}$= 9.420, P≤ 0.01; *gss*-silencing×parasitism, $F_{1,16}$= 2.748, P= 0.117; n= 5 in all bars) of non-parasitized and parasitized *P. xylostella* fourth-instar larvae. These compounds were also present in **(e)** pupae of non-parasitized *P. xylostella* and prepupae of parasitized *P, xylostella* with parasitoid inside (*gss*-silencing, $F_{1,16}$= 1.904, P= 0.186; parasitism, $F_{1,16}$= 9.844, P≤ 0.01; *gss*-silencing×parasitism, $F_{1,16}$= 0.668, P= 0.426; n= 5 in all bars). In all cases, *P. xylostella* larvae hatched upon *A. thaliana* Col-0 plants (containing aliphatic glucosinolates). Significant differences (P≤ 0.05) between means (± s.e.) were determined by Tukey HSD tests in conjunction with two-way ANOVA in **c-e**.

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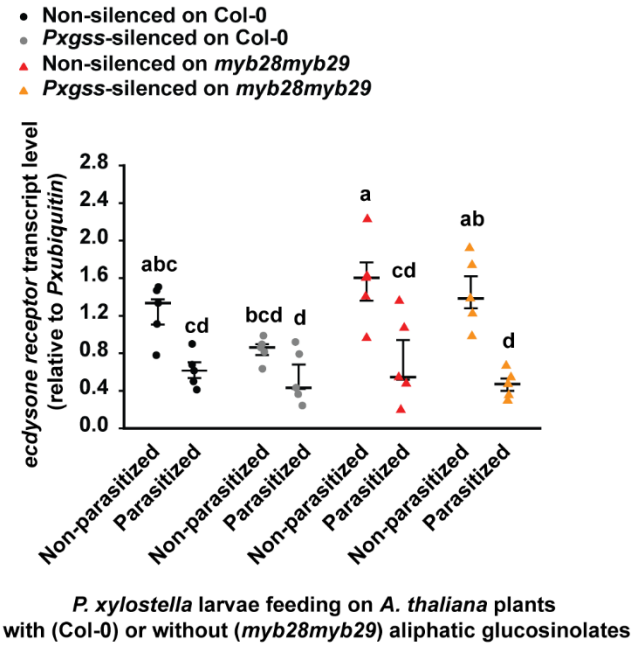

Figure S4 Expression of *P. xylostella ecdysone receptor* gene is strongly affected by parasitization by *D. semiclausum*, but not by *Pxgss* silencing or ingestion of glucosinolates. *D. semiclausum* parasitism reduced *EcR* gene expression (parasitism, $F_{1,32}$= 45.016, P≤ 0.0001; *gss*-silencing, $F_{1,32}$= 4.369, P≤ 0.05; plant, $F_{1,32}$= 5.560, P≤ 0.05; plant×parasitism, $F_{1,32}$= 5.011, P≤ 0.05; *gss*-silencing×plant×parasitism, $F_{1,32}$= 1.392, P= 0.247; n= 5 in all bars). Significant differences (P≤ 0.05) were determined by Tukey HSD tests in conjunction with multiple ANOVA.

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Table S1 External standards used for quantification.

Compounds	**Supplier**
4MSOB	Carl Roth, Karlsruhe, Germany
Desulfo-4MSOB	Obtained by incubating 4MSOB with sulfatase (Graser, Schneider, Oldham, & Gershenzon, 2000) overnight
4MSOB-ITC	BIOZOL Diagnostica Vertrieb, Eching, Germany
4MSOB-ITC-GSH	Santa Cruz Biotechnology, Dallas, TX, United States
4MSOB-ITC-CG	Synthesized as described in (Schramm, Vassão, Reichelt, Gershenzon, & Wittstock, 2012)
4MSOB-ITC-Cys	Santa Cruz Biotechnology, Dallas, TX, United States

Table S2 Primer sets for qRT-PCR validation, and corresponding gene accession numbers.

Name	Primer (5'-->3')	Gene accession
Pxecdysone receptor QF	TCAGTGCGCGATAAAGAGGA	NM001309151
Pxecdysone receptor QR	ACAGTCGAGAATCCTAGCGG	
Dsvankyrin1 QF	GTAGTACAGTGAAGCGCGTG	JI257593
Dsvankyrin1 QR	GCGATCTTTGCATCCACCTT	
Dsvankyrin2 QF	ACCGTACTACACATCGCAGT	JI257594
Dsvankyrin2 QR	CTTGAGCCAGTTGATACGCC	
Dsviral innexin1 QF	CTTGTGGCTCTGTATCGCAC	JI257597
Dsviral innexin1 QR	ACTGGCTATGGTCTCGTCAG	
Pxubiquitin QF	CGACTGATCTTCGCTGGTAAAC	NM001305519
Pxubiquitin QR	TCCTCTAAGCCTCAACACCAAG	

Graser, G., Schneider, B., Oldham, N. J., & Gershenzon, J. (2000). The methionine chain elongation pathway in the biosynthesis of glucosinolates in *Eruca sativa* (Brassicaceae). *Archives of Biochemistry and Biophysics, 378*(2), 411-419. doi:https://doi.org/10.1006/abbi.2000.1812

Schramm, K., Vassão, D. G., Reichelt, M., Gershenzon, J., & Wittstock, U. (2012). Metabolism of glucosinolate-derived isothiocyanates to glutathione conjugates in generalist lepidopteran herbivores. *Insect Biochemistry and Molecular Biology, 42*(3), 174-182. doi:https://doi.org/10.1016/j.ibmb.2011.12.002