

Supporting information for:

Different myrosinases activate sequestered glucosinolates in larvae and adults of the horseradish flea beetle

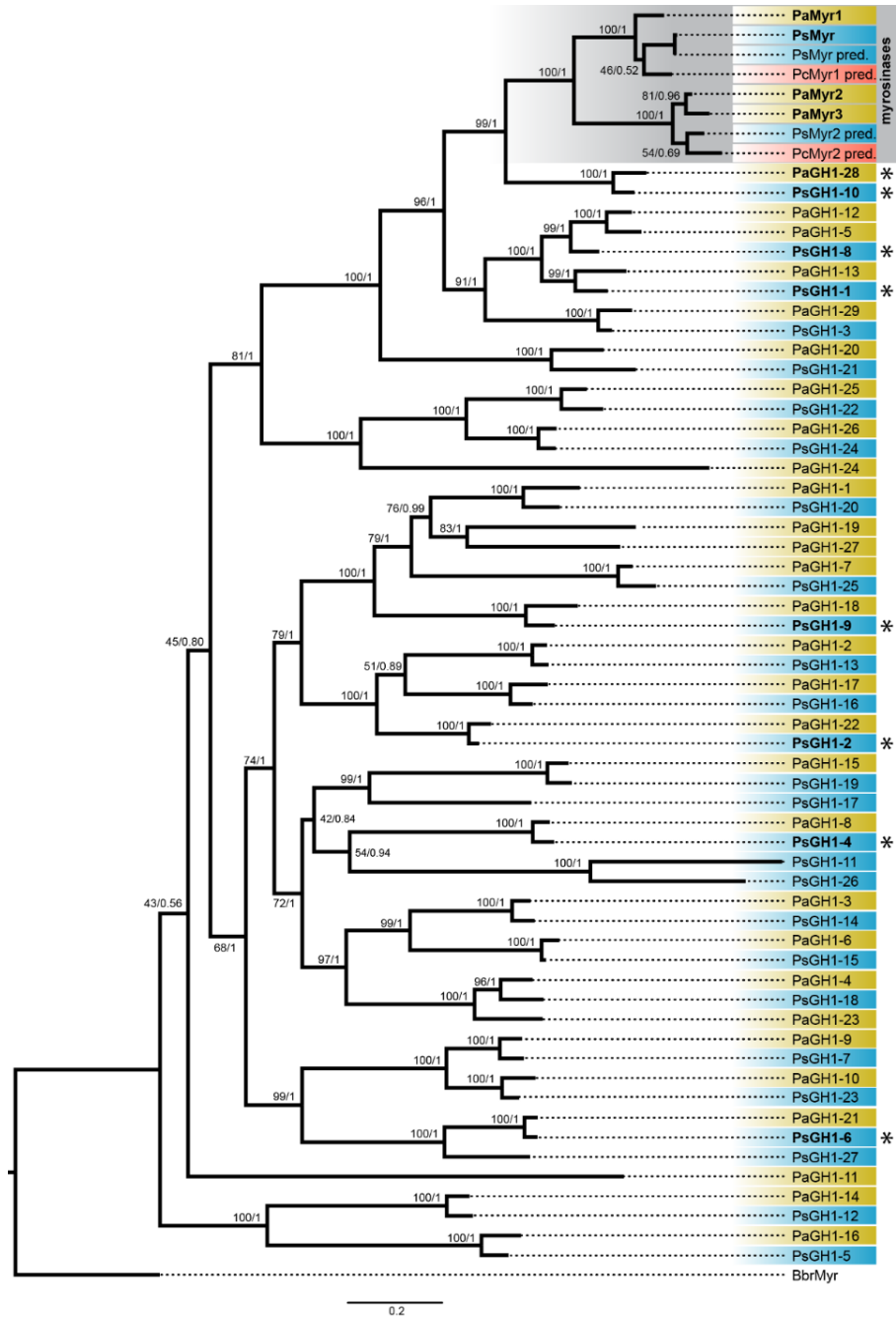


Fig. S1. Phylogeny of *Phyllotreta* GH1s. Maximum likelihood-inferred phylogeny of putative GH1 β -glucosidase enzymes from *Phyllotreta striolata* (Ps) and *Phyllotreta armoraciae* (Pa) and predicted protein sequences of myrosinase-like genes identified in genome assemblies from *P. cruciferae* (PcMyr1 pred., PcMyr2 pred.) and *P. striolata* (PsMyr pred., PsMyr2 pred.). The tree was rooted using the myrosinase from the cabbage aphid *Brevicoryne brassicae* (BbrMyr, GenBank: AAL25999.1). Bootstrap values (10,000 replicates) and posterior probability values of a Bayesian analysis using the same dataset are shown next to each node. The clade containing myrosinase and myrosinase-like enzymes is highlighted with gray background. Enzymes that were heterologously expressed and tested for enzyme activity in this study or a previous study (Beran et al., 2014) are marked with an asterisk. -, not supported in Bayesian analysis.

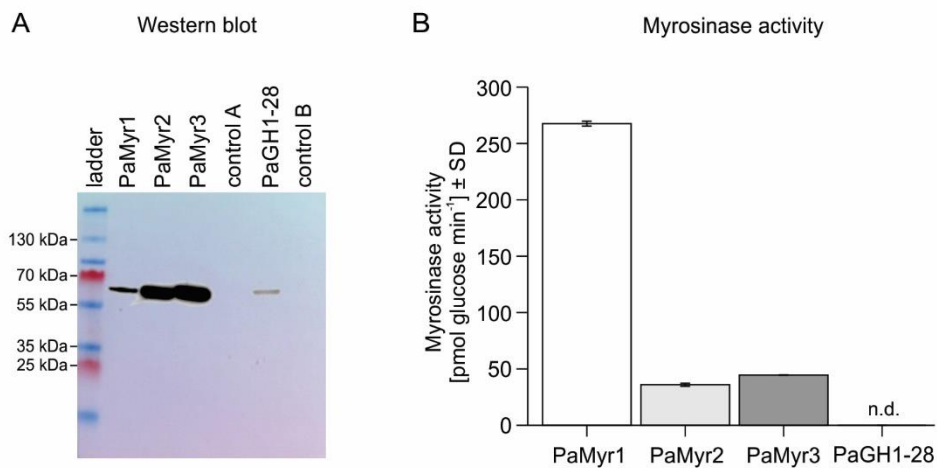


Fig. S2. Western blot analysis and myrosinase activity of recombinant enzymes. (A) Recombinant PaMyr1, PaMyr2, and PaMyr3 were detected in the cell culture medium. Recombinant PaGH1-28 was detected in the cytosol. Control A corresponds to culture medium of non-transfected cells. Control B corresponds to the cytosolic fraction of non-transfected cells. (B) Myrosinase activity of recombinant enzymes was determined in assays with dialyzed crude protein extracts using 2-propenyl glucosinolate as substrate by quantifying the amounts of released glucose. Assays were performed in triplicates. n.d., not detected.

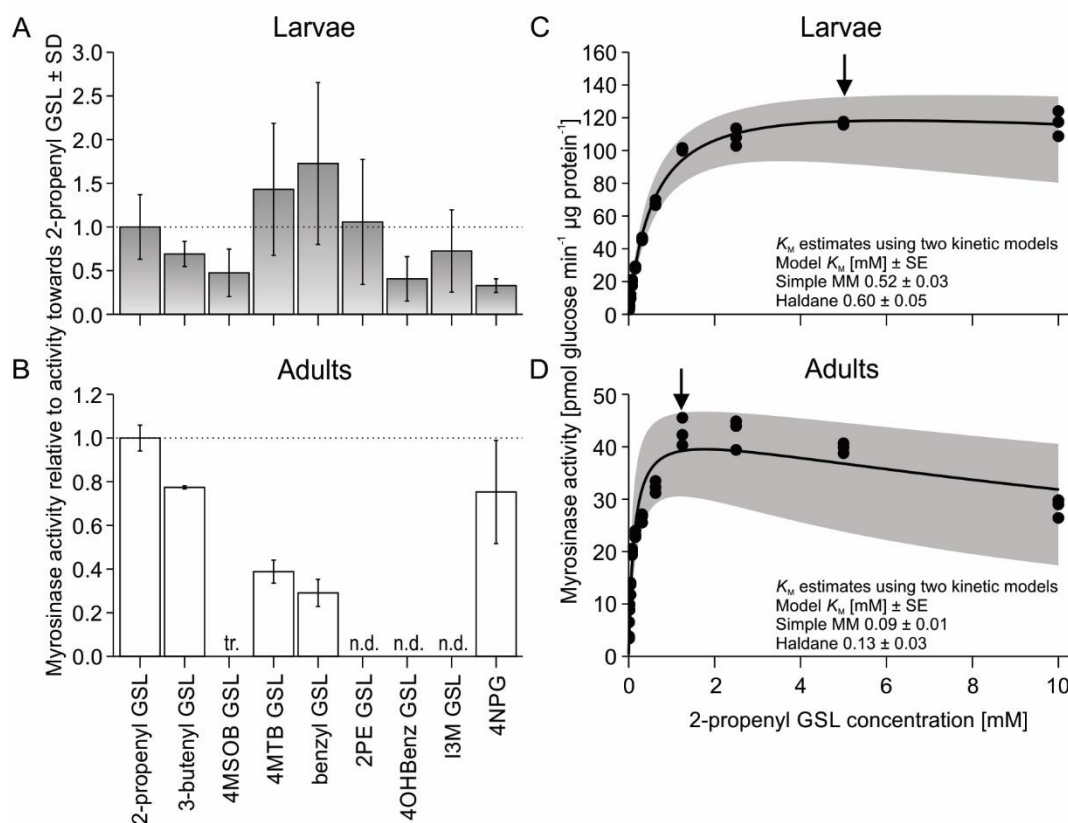


Fig. S3. Biochemical properties of myrosinase activity in crude beetle protein extracts. (A-B) Myrosinase activity towards glucosinolates (GSL) and the general β -O-glucosidase substrate 4-nitrophenyl glucopyranoside (4NPG). Substrates were tested at a substrate concentration of 0.5 mM and activity was monitored by quantifying released glucose. Enzymatic activity with different substrates is expressed relative to activity with 2-propenyl GSL, which was set to 1 (indicated with a dotted line). **(C-D)** Crude protein extracts were incubated with different concentrations of 2-propenyl GSL and activity was monitored by quantifying released glucose. K_M values of recombinant enzymes towards 2-propenyl GSL were determined based on two different equations. Lines show nonlinear regression used to determine K_M values based on the Haldane model for single-substrate inhibition ($R^2 > 0.98$ for all myrosinases). Gray bands show 95% confidence intervals. Arrows indicate the highest 2-propenyl GSL concentration used in nonlinear regression to determine the K_M values based on the Michaelis-Menten model (Simple MM). All assays were carried out in triplicates. n.d., no activity detected; 4MSOB, 4-methylsulfinylbutyl; 4MTB, 4-methylthiobutyl; 2PE, 2-phenylethyl; 4OHBenz, 4-hydroxy-benzyl; I3M, indol-3-ylmethyl.

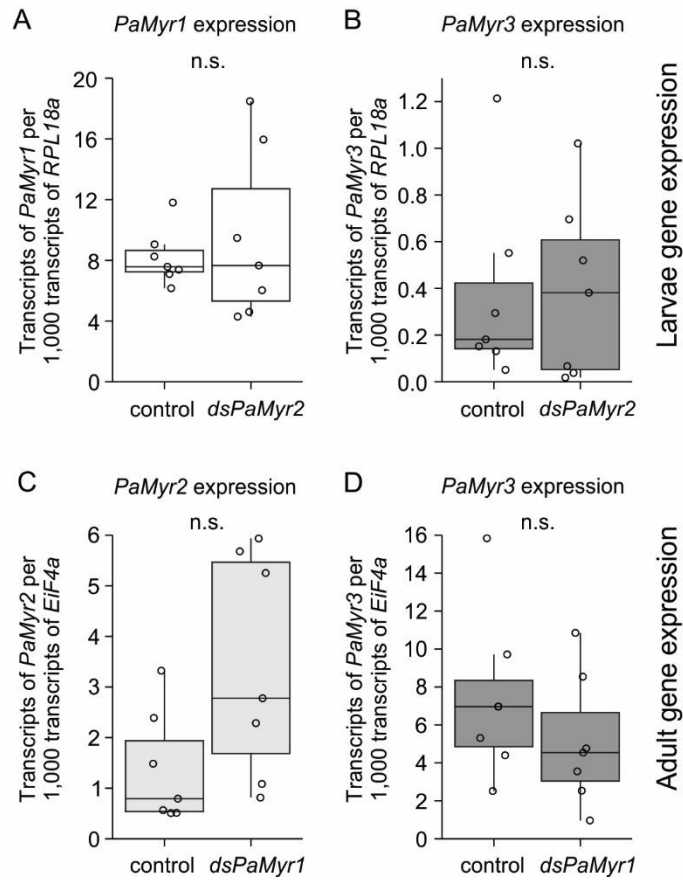


Fig. S4. Analysis of potential off-target effects of dsRNA injection in larvae and adults. The expression level of (A) *PaMyr1* and (B) *PaMyr3* did not differ between larvae injected with dsRNA targeting *PaMyr2* (*dsPaMyr2*) and control larvae (N = 7, *PaMyr1*, $t = 0.587$, $P = 0.568$; *PaMyr3*, $U = 23$, $P = 0.902$). The expression level of (C) *PaMyr2* and (D) *PaMyr3* did not differ between adults injected with dsRNA targeting *PaMyr1* (*dsPaMyr1*) and control adults (N = 7, *PaMyr2*, $U = 9$, $P = 0.053$; *PaMyr3*, $U = 17$, $P = 0.383$). Box plots show the median and interquartile range of each dataset.

Table S1. Primers used in this study

Gene	Primer name	Primer sequence 5'- 3'	Comment/purpose	primer efficiency
PaGH1-1	PaGH1-1_fl_fwd	ACGCATTAACGCGAATTA AAAATGT	Cloning in pCR4-TOPO vector for sequencing; fwd	
PaGH1-1	PaGH1-1_fl_rev	TTATAAAATGTAATTCTCAAATATTCGATT	Cloning in pCR4-TOPO vector for sequencing; rev	
PaGH1-1	PaGH1-1_fl_iseq	AGGCTGGACCAACCCGCGA	internal sequencing	
PaGH1-2	PaGH1-2_fl_fwd	GTGGTTTAATCATTGCGATCCATA	Cloning in pCR4-TOPO vector for sequencing; fwd	
PaGH1-2	PaGH1-2_fl_rev	TTAATATGAGATAACATGAGTGCATAA	Cloning in pCR4-TOPO vector for sequencing; rev	
PaGH1-2	PaGH1-2_fl_iseq	CAAGACGCCGGCGGTTG	internal sequencing	
PaGH1-3	PaGH1-3_fl_fwd	CACGCGAGCATCGCATATC	Cloning in pCR4-TOPO vector for sequencing; fwd	
PaGH1-3	PaGH1-3_fl_rev	TATATTCTCTTCAGGTCACAAGGA	Cloning in pCR4-TOPO vector for sequencing; rev	
PaGH1-3	PaGH1-3_fl_iseq	CGGCATATCTCCGTTTCGTAAC	internal sequencing	
PaGH1-4	PaGH1-4_fl_fwd	TGAGCAGCTATCACGTTGAAGA	Cloning in pCR4-TOPO vector for sequencing; fwd	
PaGH1-4	PaGH1-4_fl_rev	AGTTGCATACTGACTTGAGCATT	Cloning in pCR4-TOPO vector for sequencing; rev	
PaGH1-4	PaGH1-4_fl_iseq	CCTGAACCCGTCGGTGG	internal sequencing	
PaGH1-5	PaGH1-31_fl_fwd	CAGTTGTCAGAACACAATAATTGTT	Cloning in pCR4-TOPO vector for sequencing; fwd	
PaGH1-5	PaGH1-31_fl_rev	CAATTTATTTCGATAACTGACTGTTG	Cloning in pCR4-TOPO vector for sequencing; rev	
PaGH1-5/PaGH1-12	PaGH1-12_fl_iseq	CTGTTCGATATTCCTACGGAAC	internal sequencing PaGH1-5 and PaGH1-12	
PaGH1-6	PaGH1-6_fl_fwd	ACACTGTTTCAACAAAGTCGATAATA	Cloning in pCR4-TOPO vector for sequencing; fwd	
PaGH1-6	PaGH1-6_fl_rev	CATACGTTTATTACAGTCGTAATGT	Cloning in pCR4-TOPO vector for sequencing; rev	
PaGH1-6	PaGH1-6_fl_iseq	TGGTGGATAACCTTCAACGAG	internal sequencing	
PaGH1-7	PaGH1-7_fl_fwd	GAGGTGACAACAAGCTAAAATTACA	Cloning in pCR4-TOPO vector for sequencing; fwd	
PaGH1-7	PaGH1-7_fl_rev	ACGAATGCAAAGAAGATGGGGTG	Cloning in pCR4-TOPO vector for sequencing; rev	
PaGH1-7	PaGH1-7_fl_iseq	CTCGCTCCAGGAGTTTGTTC	internal sequencing	
PaGH1-8	PaGH1-8_fl_fwd	AATGCTCCTTACTTGCAACTTA	Cloning in pCR4-TOPO vector for sequencing; fwd	
PaGH1-8	PaGH1-8_fl_rev	AAAGGAGATCAAAGAACAACGCT	Cloning in pCR4-TOPO vector for sequencing; rev	
PaGH1-8	PaGH1-8_fl_iseq	AACTTCGCCAACGATGTGGAC	internal sequencing	
PaGH1-9	PaGH1-9_fl_fwd	ACACCACAATCTGATAGATAAGCA	Cloning in pCR4-TOPO vector for sequencing; fwd	
PaGH1-9	PaGH1-9_fl_rev	GAGTAAACAGTCGCAGGAAATTG	Cloning in pCR4-TOPO vector for sequencing; rev	
PaGH1-9	PaGH1-9_iseq	ACGCCAAGTTCGCGTTCGA	internal sequencing	
PaGH1-10	PaGH1-10_fl_g_fwd	CTACAGATAAGTACCTAATTTTGGT	Cloning in pCR4-TOPO vector for sequencing; fwd	
PaGH1-10	PaGH1-10_fl_rev	CGGATGGTTTCGGTGAATAATG	Cloning in pCR4-TOPO vector for sequencing; rev	
PaGH1-10	PaGH1-10_fl_iseq	CTGGTGGATCACCGTCAACG	internal sequencing	

PaGH1-11	PaGH1-11_fl_fwd	TGCATCTTGGTTCTTCTAGTTCTAT	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-11	PaGH1-11_fl_rev	TACCAGAGAGTAGGATGCTAAGA	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-11	PaGH1-11_iseq	TGGGTGACAATTTACCATTGGGA	internal sequencing
PaGH1-12	PaGH1-12_fl_fwd	AAGGTGCCTGGGAGAGTTGT	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-12	PaGH1-12_fl_rev	TCCACATAATAAAGGTTAGGTTCGA	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-13	PaGH1-13_fl_g_fwd	CTAGCCTAAAAATTGGGAAGGG	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-13	PaGH1-13_fl_g_rev	CATTATACATGAGGTAAGCCTTGG	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-13	PaGH1-13_fl_iseq	CCGGAAGTCGGGGCTTCT	internal sequencing
PaGH1-13	PaGH1-13-fl-iseq2	GACCGGTGATATCGCTTGTG	internal sequencing
PaGH1-14	PaGH1-14_fl_fwd	ACAGTTTACTGTAATACACATCAGAT	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-14	PaGH1-14_fl_rev	ACGACGAGGAGTTCAGGACT	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-14	PaGH1-14_fl_iseq	TATATTCCTGACCTACCTTCCAG	internal sequencing
PaGH1-15	PaGH1-15_fl_fwd	ACCTCGTATGACAATTTACACGT	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-15	PaGH1-15_fl_rev	CACCTTATAATCCTCACGAGATC	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-15	PaGH1-15_fl_iseq	CGAGCCGTACGAAACGTGC	internal sequencing
PaGH1-16	PaGH1-16_fl_fwd	GAACTGTATAAATACTCGCTTATTG	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-16	PaGH1-16_fl_rev	GCTACATTATTATGTATGTAGGTATTA	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-16	PaGH1-16_fl_iseq	TGCCGGTTACGCTGAGGTT	internal sequencing
PaGH1-17	PaGH1-17_fl_fwd	CCCAATTGTGGTTTATCTAATCGAA	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-17	PaGH1-17_fl_rev	AGGTATCAACAGGTTAATTTGCTAG	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-17	PaGH1-17_fl_iseq	GCCTTTGCAAGACATTGGCG	internal sequencing
PaGH1-18	PaGH1-18_fl_fwd	ACCTGGTAACAACGTCGGTAA	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-18	PaGH1-18_fl_rev	ACCAAAACACAATTCATCGACTACT	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-18	PaGH1-18_fl_iseq	TCGTCCGATGGTACTCCGA	internal sequencing
PaGH1-19	PaGH1-19_fl_fwd	CACTCTGTATTTGCACATAATTATCA	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-19	PaGH1-19_fl_rev	AGTGCGGAGGAAGGGGAT	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-19	PaGH1-19_fl_iseq	CGACGTGAAGCATTGGATGAC	internal sequencing
PaGH1-20	PaGH1-34_fl_g_fwd	GGAAGTATTTATATATGTTATTGAGGT	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-20	PaGH1-34_fl_g_rev	CAGTTTACCAGTGCATAAGAG	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-20	PaGH1-34_fl_iseq	CCAGAAGTAGGCATATGGATTC	internal sequencing
PaGH1-21	PaGH1-21_fl_g_fwd	GTTCATAAGTATATAAGATACCTATGTA	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-21	PaGH1-21_fl_g_rev	CAACACCGCAACAGGTTGG	Cloning in pCR4-TOPO vector for sequencing; rev

PaGH1-21	PaGH1-21_fl_iseq	CGGACATCGTCGATTGGTTC	internal sequencing
PaGH1-22	PaGH1-22_fl_fwd	AACTTGGAAGAGCAGGTATCGAA	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-22	PaGH1-22_fl_rev	ATGTAATTAATAGATAAAATCTAACTGCT	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-22	PaGH1-22_fl_rev2	CTAACTGCTTTAGTCGGTGCAA	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-22	PaGH1-22_fl_iseq	TGGCTGACATTCAACGAACCCA	internal sequencing
PaGH1-23	PaGH1-23_fl_fwd	ACCAAATTCGTCATAGTTTCGT	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-23	PaGH1-23_fl_rev	TGCTCTTGGGACAAATTATAAGCA	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-23	PaGH1-23_fl_iseq	ACGCTCTTCGGGGACGAC	internal sequencing
PaGH1-24	PaGH1-24_fl_g_fwd	CTTGTGTGTAGTGTCTCCAG	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-24	PaGH1-24_fl_rev	CATAATTTGCTGTAGAAAAGAATATCAG	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-24	PaGH1-24_fl_iseq	CTACACCGACAACGTGTTCCA	internal sequencing
PaGH1-25	PaGH1-25_fl_g_fwd	ATTATTGCAGGTTTTGTAGCTAGATA	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-25	PaGH1-25_fl_g_rev	CACGACGGTCAAAAACTGCA	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-25	PaGH1-25_fl_iseq	GATCACCATAAACGAACCTAGAC	internal sequencing
PaGH1-26	PaGH1-26_fl_g_fwd	AGGAGCATGATAAATAATTGTAATCTT	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-26	PaGH1-26_fl_rev	GCAACGTTTGATTTAATCTTGGGT	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-26	PaGH1-26_fl_iseq	CAGGATCTGGGCGGTTGG	internal sequencing
PaGH1-27	PaGH1-27_fl_fwd	AGCGCGGGTACTACTTATAGA	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-27	PaGH1-27_fl_rev	TACATTTTCGATCGAT TTGGTCGA	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-27	PaGH1-27_fl_iseq	GACCGACGAGCAAATAGTCCA	internal sequencing
PaGH1-28	PaGH1-28_fl_g_fwd	GACTCTTACAAGTGCTGTGTCTA	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-28	PaGH1-28_fl_g_rev	ATCACTATATTACAACCTATACATCACTA	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-28	PaGH1-28_fl_iseq	CGACGGGATCGCCGACTA	internal sequencing
PaGH1-29	PaGH1-38_fl_g_fwd	CAGTGGGGAGCCGAAGTAC	Cloning in pCR4-TOPO vector for sequencing; fwd
PaGH1-29	PaGH1-38_fl_g_rev	CCCATATGCGTTGCTGATTAGA	Cloning in pCR4-TOPO vector for sequencing; rev
PaGH1-29	PaGH1-38_fl_iseq	CTTTCCCGGAAGTCAGTATTTG	internal sequencing
PaMyr2	Parm_C41_Seq_fwd	TGAGGTCCCTACGGTGACGGAAAGCA	Sequencing PaMyr2
PaMyr2	PaMyrIIa-5'R-1	CTCTTTGTGCTTGTTCACCAACATGGTGGTGT	5'RACE PCR
PaMyr3	PaMyrIIb-5'R-1	TTCTACACCGTTGGCTACCAACGCGGAATAGTA	5'RACE PCR
PaMyr3	PaMyrIIb-5'R-2	ATGATTAAGCAGCTTTCTAGCACCGTCTGGGA	5'RACE PCR
PaMyr1	Parm_C2654_BamHI_fw	TGGATCCCATGCAGCAAAAAATAGCATTCG	cloning into pIEx-4 expression vector without stop codon; fwd
PaMyr1	Parm_C2654_NotI_rev	TGCGGCCGCCTTTACGTTAGCGCAATTTATACGTT	cloning into pIEx-4 expression vector without stop codon; rev

PaMyr2/PaMyr3	Parm_C41_BamHI_fw	TGGATCCCATGGCGAAACAAATCCCATTACTCT	cloning into pIEx-4 expression vector without stop codon; fwd
PaMyr2	Parm_C41_NotI_rev	TGCGGCCGCCTCCAGGTCATCACATGTAAACCT	cloning into pIEx-4 expression vector without stop codon; rev
PaMyr3	PaMyrIIb_NotI_rev	TGCGGCCGCCTCAACGTCTTCACATGATAACCTGTA	cloning into pIEx-4 expression vector without stop codon; rev
PaGH1-28	PaGH1-28_BamHI_fwd	TGGATCCCATGTTTCTCAAATTAGTTATAACCTTAAACT	cloning into pIEx-4 expression vector without stop codon; fwd
PaGH1-28	PaGH1-28_NotI_rev	TGCGGCCGCTAAAATTTGATTATCATTCCACACAGAG	cloning into pIEx-4 expression vector without stop codon; rev
PaGH1-20	PaGH1-34_BamHI_fwd	TGGATCCCATGGGTGTTTACGTGCATTTATCA	cloning into pIEx-4 expression vector without stop codon; fwd
PaGH1-20	PaGH1-34_NotI_rev	TGCGGCCGCACAGGCTGTTCTACGATTGAAAC	cloning into pIEx-4 expression vector without stop codon; rev
PaMyr1	T7-PaMYR-F1	TAATACGACTCACTATAGGGAGAGGAGCAAGAAAATTACTGAACCAT	Amplification of DNA templates for dsRNA synthesis; fwd
PaMyr1	T7-PaMYR-R1	TAATACGACTCACTATAGGGAGAAAAATGTAGCAGAAAT	Amplification of DNA templates for dsRNA synthesis; rev
PaMyr2	T7_PaMyrIIa_fwd	ATCCTAATACGACTCACTATAGGTACATTTTGAAGCCATGCAGGT	Amplification of DNA templates for dsRNA synthesis; fwd
PaMyr2	T7_PaMyrIIa_rev	ATCCTAATACGACTCACTATAGGTCACTCCAGGTCATCACATGTTA	Amplification of DNA templates for dsRNA synthesis; rev
IMPI	T7-IMPI-F2	TAATACGACTCACTATAGGGAGAGTAATGACAAGTGCTACTGTGAAGAT	Amplification of DNA templates for dsRNA synthesis; fwd
IMPI	T7-IMPI-R2	TAATACGACTCACTATAGGGAGAGGGGAGTCAATGCAGGAAAAC	Amplification of DNA templates for dsRNA synthesis; rev
RPL18a	qRPL18a-F2	GGCCACAAGTCAAACAATTCCA	qPCR; fwd
RPL18a	qRPL18a-R2	AAGTAAGTTCTGGGTTTGC	qPCR; rev
RPL32e	qRPL32e-2F	ATACTGTGCTGAAATCGCCCAT	qPCR; fwd
RPL32e	qRPL32e-2R	AATCTAGCGTGTCCATTGGTGA	qPCR; rev
EiF4a	qPaEiF4a_F	CACGGTGACATGGAGCAAAG	qPCR; fwd
EiF4a	qPaEiF4a_R	ACCTCTGGCCAACAAATCGG	qPCR; rev
RPS4e	qRPS4e-F	CGTATTACTGCTGAAGAAGC	qPCR; fwd
RPS4e	qRPS4e-R	ATCGTGGGTCACCAAGAACG	qPCR; rev
PaMyr1	qPaC2654-3-F	AACGGTTACGCTGACACGAT	qPCR; fwd
PaMyr1	qPaC2654-3-R	ACCGCCACCATTCCGTATTT	qPCR; rev
PaMyr2	Parm_C41_qPCR_2_F	ACACCAGAGCTGTGATGTCG	qPCR; fwd
PaMyr2	Parm_C41_qPCR_2_R	ATCTCTGACACCACTCGGGA	qPCR; rev
PaMyr3	q-PaMyrIIb-3-fwd	ACTATTCCGCGTTGGTAGCC	qPCR; fwd
PaMyr3	q-PaMyrIIb-3-rev	CAGGATGGTTCGACGTCACA	qPCR; rev
PaGH1-28	q-PaGH1-28-3-F	TTCCTGCGTTACGCTCTGAA	qPCR; fwd
PaGH1-28	q-PaGH1-28-3-R	AGCTCGTCTCGTTGTAAGGC	qPCR; rev

Table S2. Summary of putative GH1 β -glucosidases identified in *P. armoraciae* transcriptomes

Gene	GenBank accession number	length protein	number signal peptides	length protein without predicted signal peptide	Isoelectric point	MW [kDa]	Number of predicted N-glycosylation sites
<i>PaMyr1</i>	OP313699	495	19	476	6.19	54.7	1
<i>PaMyr2</i>	OP313700	494	20	474	4.97	54.6	2
<i>PaMyr3</i>	OP313701	494	20	474	4.93	54.5	3
<i>PaGH1-1</i>	OP313702	493	18	475	6.30	55.1	2
<i>PaGH1-2</i>	OP313703	518	23	495	5.87	57.7	2
<i>PaGH1-3</i>	OP313704	492	17	475	4.94	54.0	3
<i>PaGH1-4</i>	OP313705	495	21	474	6.38	54.6	5
<i>PaGH1-5</i>	OP313706	539	20	519	6.98	60.5	4
<i>PaGH1-6</i>	OP313707	491	16	475	5.62	54.5	4
<i>PaGH1-7</i>	OP313708	478	0	478	6.17	55.0	1
<i>PaGH1-8</i>	OP313709	487	19	468	5.20	53.5	4
<i>PaGH1-9</i>	OP313710	501	18	483	5.59	55.1	4
<i>PaGH1-10</i>	OP313711	494	16	478	4.84	54.5	4
<i>PaGH1-11</i>	OP313712	480	18	462	8.04	53.1	4
<i>PaGH1-12</i>	OP313713	539	20	519	8.39	60.8	4
<i>PaGH1-13</i>	OP313714	516	20	496	8.74	57.2	5
<i>PaGH1-14</i>	OP313715	493	20	473	6.58	54.8	5
<i>PaGH1-15</i>	OP313716	477	17	460	4.93	53.2	5
<i>PaGH1-16</i>	OP313717	469	0	469	6.34	53.6	1
<i>PaGH1-17</i>	OP313718	495	18	477	5.27	54.9	1
<i>PaGH1-18</i>	OP313719	498	18	480	5.43	55.2	4
<i>PaGH1-19</i>	OP313720	501	20	481	6.80	55.3	5
<i>PaGH1-20</i>	OP313721	517	22	495	8.94	57.2	6
<i>PaGH1-21</i>	OP313722	507	23	484	5.22	55.7	4
<i>PaGH1-22</i>	OP313723	494	17	477	6.01	54.8	2
<i>PaGH1-23</i>	OP313724	496	21	475	5.04	54.2	4
<i>PaGH1-24</i>	OP313725	606	21	585	5.07	65.4	7
<i>PaGH1-25</i>	OP313726	553	22	531	5.05	60.1	7

<i>PaGH1-26</i>	OP313727	533	20	513	5.43	58.3	5
<i>PaGH1-27</i>	OP313728	495	16	479	4.91	55.1	3
<i>PaGH1-28</i>	OP313729	504	18	486	7.37	56.5	3
<i>PaGH1-29</i>	OP313730	524	22	502	6.05	58.9	4
<i>PsMyr (from genome)</i>	-	495	20	475	5.82	54.9	3
<i>PsMyr2 (from genome)</i>	-	494	20	474	5.04	54.4	1
<i>PcMyr1 (from genome)</i>	-	494	20	474	5.96	54.9	1
<i>PcMyr2 (from genome)</i>	-	495	20	475	5.08	54.6	1

Table S3. Results of statistical analyses

Experiment	Comparison	Statistical Method	Variance structure	Variable	Transformation	<i>N</i>	Statistics	<i>P</i>	
Candidate gene expression and myrosinase activity in different life-stages	gene expression of the three myrosinases	Generalized least squares method	varComb (varIdent (form = ~ 1 life-stage), form = ~ 1 gene))	myrosinase gene	log	6	<i>LR</i> = 48.65	< 0.001	
				life-stage			<i>LR</i> = 37.48	< 0.001	
				Interaction			<i>LR</i> = 284.54	< 0.001	
		<i>PaMyr1</i> gene expression	Generalized least squares method	varIdent (form = ~ 1 life-stage)	life-stage	log	6	<i>LR</i> = 140.61	< 0.001
		<i>PaMyr2</i> gene expression	Generalized least squares method	varIdent (form = ~ 1 life-stage)	life-stage	log	6	<i>LR</i> = 85.44	< 0.001
		<i>PaMyr3</i> gene expression	Generalized least squares method	varIdent (form = ~ 1 life-stage)	life-stage	log	6	<i>LR</i> = 36.86	< 0.001
	myrosinase activity per mg fresh weight	Generalized least squares method	varIdent (form = ~ 1 life-stage)	life-stage	-	6-7	<i>LR</i> = 101.47	< 0.001	
	<i>PaGHI-28</i> gene expression	Generalized least squares method	varIdent (form = ~ 1 life-stage)	life-stage	log	6	<i>LR</i> = 59.95	< 0.001	
RNA interference in adult beetles	<i>PaMyr1</i> gene expression	Mann-Whitney rank sum test	-		-		<i>U</i> = 0	< 0.001	
	<i>PaMyr2</i> gene expression	Mann-Whitney rank sum test	-	dsRNA treatment	-	7	<i>U</i> = 9	0.058	
	<i>PaMyr3</i> gene expression	Mann-Whitney rank sum test	-		-		<i>U</i> = 17	0.383	
	myrosinase activity per mg fresh weight	Two-tailed Student's <i>t</i> -test	-	dsRNA treatment	-	8	<i>t</i> = 5.140	< 0.001	
	2-propenyl glucosinolate amount per mg fresh weight	Two-tailed Student's <i>t</i> -test	-		-	12	<i>t</i> = 1.192	0.246	
RNA interference in larvae	<i>PaMyr1</i> gene expression	Two-tailed Student's <i>t</i> -test	-		-		<i>t</i> = 0.587	0.568	
	<i>PaMyr2</i> gene expression	Two-tailed Student's <i>t</i> -test	-	dsRNA treatment	-	7	<i>t</i> = 2.528	0.027	
	<i>PaMyr3</i> gene expression	Mann-Whitney rank sum test	-		-		<i>U</i> = 23	0.902	
	myrosinase activity per mg fresh weight	Two-tailed Student's <i>t</i> -test	-	dsRNA treatment	-	8	<i>t</i> = 5.556	< 0.001	
	2-propenyl glucosinolate amount per mg fresh weight	Two-tailed Student's <i>t</i> -test	-		-	7	<i>t</i> = 2.492	0.028	
Predation experiment	<i>PaMyr1</i> gene expression	Two-tailed Student's <i>t</i> -test	-		-		<i>t</i> = 0.272	0.78	
	<i>PaMyr2</i> gene expression	Mann-Whitney rank sum test	-	dsRNA treatment	-	7	<i>U</i> = 7	0.026	
	<i>PaMyr3</i> gene expression	Mann-Whitney rank sum test	-		-		<i>U</i> = 11	0.097	
	myrosinase activity per mg fresh weight	Mann-Whitney rank sum test	-	dsRNA treatment	-	8	<i>U</i> = 2	< 0.001	
	<i>P. armoraciae</i> larval survival	Log-rank-test	-	dsRNA treatment experiment day	-	68-72	$\chi^2 = 21.8$ $\chi^2 = 4.5$	< 0.001 0.107	

Feeding experiment larvae	<i>PaMyr1</i> gene expression	Two-tailed Student's <i>t</i> -test	-	-	6-7	$t = 0.806$	0.555
	<i>PaMyr2</i> gene expression	Mann-Whitney rank sum test	-	dsRNA treatment	6-7	$U = 5$	0.022
	<i>PaMyr3</i> gene expression	Mann-Whitney rank sum test	-	-	6-7	$U = 19$	0.836
	myrosinase activity per mg fresh weight	Mann-Whitney rank sum test	-	dsRNA treatment	7	$U = 5$	0.011
	proportion of 4MSOB GSL hydrolysis products in larval feces	Two-tailed Student's <i>t</i> -test	-	dsRNA treatment	12	$t = 0.248$	0.804
	proportion of 4MSOB GSL hydrolysis products in larval bodies	Mann-Whitney rank sum test	-	dsRNA treatment	12	$U = 30$	0.017

Table S4. *In silico* off-target prediction of the dsRNA designs against the local *P. armoraciae* transcriptome databases

Target gene	Hit	1-mismatch count	2-mismatch count	Sequence annotation
<i>IMPI</i> (control)	<i>Parm_LP_c4</i>	0	1	cytochrome c oxidase assembly protein COX15 homolog
	<i>Parm_LP_c9054</i>	0	1	zinc finger protein 888-like
	<i>Parm_LP_c9294</i>	0	1	beta-alanine-activating enzyme-like isoform X1
	<i>Parm_LP_c14962</i>	0	1	5-hydroxytryptamine receptor 2C-like
	<i>Parm_GBB_C2694</i>	0	1	unnamed protein product [Brassicogethes aeneus]
	<i>Parm_GBB_C6885</i>	0	1	poly [ADP-ribose] polymerase
	<i>Parm_GBB_C8370</i>	0	1	beta-alanine-activating enzyme-like isoform X2
	<i>Parm_GBB_C10195</i>	0	1	5-hydroxytryptamine receptor 2C-like
	<i>Parm_GBB_C12996</i>	0	1	zinc finger protein 888-like
	<i>Parm_GBB_C19622</i>	0	1	LOW QUALITY PROTEIN: uncharacterized protein LOC115885494
	<i>Parm_GBB_C25101</i>	0	1	unnamed protein product
	<i>Parm_GBB_C27866</i>	0	1	marc-1
	<i>Parm_BB_C2192</i>	0	1	cytochrome c oxidase assembly protein COX15 homolog
	<i>Parm_BB_C10503</i>	0	1	microcephalin
	<i>Parm_BB_C20334</i>	0	1	zinc finger protein 888-like
<i>Parm_BB_C27814</i>	0	1	dopamine D2-like recepto	
<i>PaMyr1</i>	<i>Parm_LP_c688</i>	0	4	beta-1,3-glucan-binding protein-like
	<i>Parm_LP_c693</i>	0	1	PaMyr3
	<i>Parm_LP_c2284</i>	0	1	uncharacterized protein LOC114342259
	<i>Parm_LP_c2748</i>	0	1	fatty acid synthase-like
	<i>Parm_LP_c6853</i>	0	1	uncharacterized protein LOC114333505
	<i>Parm_GBB_C36714</i>	1	2	No Hit
	<i>Parm_GBB_C4530</i>	0	1	uncharacterized protein LOC114333505
	<i>Parm_GBB_C22037</i>	0	1	PaMyr3
	<i>Parm_GBB_C34896</i>	0	1	fatty acid synthase-like
	<i>Parm_BB_C28599</i>	1	2	No Hit
	<i>Parm_BB_C1424</i>	0	1	fatty acid synthase-like
	<i>Parm_BB_C2373</i>	0	1	death-inducer obliterator 1-like
	<i>Parm_BB_C3804</i>	0	1	uncharacterized protein LOC114342259
	<i>Parm_BB_C21049</i>	0	1	No Hit
	<i>Parm_BB_C27579</i>	0	1	No Hit
<i>PaMyr2</i>	<i>Parm_LP_c693</i>	41	38	PaMyr3
	<i>Parm_LP_c6174</i>	0	2	No Hit
	<i>Parm_LP_c8465</i>	0	2	PaGH1-28
	<i>Parm_LP_c495</i>	0	1	microtubule-associated serine/threonine-protein kinase 3 isoform X2
	<i>Parm_LP_c3092</i>	0	1	PaGH1-16
	<i>Parm_LP_c9501</i>	0	1	probable proline--tRNA ligase, mitochondrial
	<i>Parm_LP_c11552</i>	0	1	hyaluronidase-like
	<i>Parm_GBB_C22037</i>	41	38	PaMyr3
	<i>Parm_GBB_C653</i>	0	4	PaGH1-18
	<i>Parm_GBB_C36642</i>	0	2	PaGH1-28
<i>Parm_GBB_C324</i>	0	1	PaGH1-16	

<i>Parm_GBB_C1484</i>	0	1	probable proline--tRNA ligase, mitochondrial
<i>Parm_GBB_C9477</i>	0	1	microtubule-associated serine/threonine-protein kinase 3 isoform X2
<i>Parm_GBB_C16713</i>	0	1	hyaluronidase-like
<i>Parm_GBB_C22656</i>	0	1	Glucose-6-phosphate 1-dehydrogenase
<i>Parm_GBB_C22858</i>	0	1	unnamed protein product
<i>Parm_GBB_C24664</i>	0	1	unnamed protein product
<i>Parm_GBB_C25866</i>	0	1	hypothetical protein AAVH_02602
<i>Parm_GBB_C37647</i>	0	1	No Hit
<i>Parm_GBB_C38976</i>	0	1	lysophosphatidylcholine acyltransferase isoform X2
<i>Parm_BB_C15344</i>	59	33	misassembled contig of PaMyr2 and PaMyr3
<i>Parm_BB_C12167</i>	0	4	PaGH1-18
<i>Parm_BB_C21485</i>	0	3	40S ribosomal protein S2-like
<i>Parm_BB_C8334</i>	0	2	PaGH1-28
<i>Parm_BB_C218</i>	0	1	probable proline--tRNA ligase, mitochondrial
<i>Parm_BB_C2766</i>	0	1	microtubule-associated serine/threonine-protein kinase 3 isoform X2
<i>Parm_BB_C7049</i>	0	1	dopamine N-acetyltransferase-like
<i>Parm_BB_C9210</i>	0	1	hyaluronidase-like

Table S5. LC-MS/MS parameters for multiple reaction monitoring (MRM)

Analyte	Q1 [m/z]	Q3 [m/z]	DP [V]	CE [V]
4MSOB GSL	435.9	95.8	-65	-60
4MSOB-isothiocyanate	178.11	114	26	13
4MSOB-cyanide	146	129	38	13
4MSOB-amin	136	72	26	17

CE, collision energy; DP, declustering potential

Table S6. Myrosinase gene expression and activity in larvae used in predation and feeding assays

Experiment	<i>N</i>	dsRNA treatment	transcripts of <i>PaMyr1</i> per 1,000 transcripts of <i>RPS4e</i> mean±sd	transcripts of <i>PaMyr2</i> per 1,000 transcripts of <i>RPS4e</i> mean±sd	transcripts of <i>PaMyr3</i> per 1,000 transcripts of <i>RPS4e</i> mean±sd	<i>N</i>	Activity [pmol glucose min ⁻¹ mg FW ⁻¹] mean±sd
RNAi predation	7	<i>dsIMPI</i>	0.5±0.5	1524.5±836.7	66.6±169.1	8	1251.2±544.8
	7	<i>dsPaMyr2</i>	0.5±0.3	501.7±539.1	260.1±301.9	8	219.1±232.1
RNAi feeding	7	<i>dsIMPI</i>	1.6±1.5	1497.2±819.3	79.2±132.0	7	1713.3±1150.7
	6	<i>dsPaMyr2</i>	1.2±0.8	413.3±362.8	141.6±200.4	7	481.1±485.4

RPS4e, 40S ribosomal protein subunit 4.

Table S7. Glucosinolate concentration in *B. rapa* leaves and *P. armoraciae* reared on *B. rapa*

Glucosinolate (GSL)	Mean GSL concentration [nmol GSL × mg ⁻¹ fresh weight ± SD]				Statistical method	Statistics	P
	<i>B. rapa</i> (N = 6)	Third instar larvae (N = 20)	Pupa (N = 20)	Adults (N = 20)			
3-Butenyl GSL	0.15 ± 0.14	0.03 ± 0.07 b	0.10 ± 0.21 b	2.22 ± 0.91 a	Kruskal-Wallis one way ANOVA	H = 43.777	≤ 0.001
4-Pentenyl GSL	0.24 ± 0.16	0.07 ± 0.08 b	0.26 ± 0.28 b	2.45 ± 1.04 a	Kruskal-Wallis one way ANOVA	H = 43.671	≤ 0.001
2OH3But GSL	0.24 ± 0.11	1.50 ± 0.66 b	2.27 ± 1.03 b	3.79 ± 1.04 a	one way ANOVA	F = 31.512	≤ 0.001
2OH4Pent GSL	0.03 ± 0.02	0.12 ± 0.08 c	0.26 ± 0.15 b	0.63 ± 0.31 a	Kruskal-Wallis one way ANOVA	H = 37.968	≤ 0.001
5MSOP GSL	0.10 ± 0.12	0.79 ± 0.23 b	1.06 ± 0.46 a	1.18 ± 0.03 a	one way ANOVA	F = 6.495	= 0.003
Benzyl GSL	0.08 ± 0.05	0.07 ± 0.02 b	0.09 ± 0.04 b	0.48 ± 0.12 a	Kruskal-Wallis one way ANOVA	H = 39.671	≤ 0.001
2PE GSL	0.02 ± 0.01	0.02 ± 0.06 b	0.10 ± 0.13 b	0.31 ± 0.12 a	Kruskal-Wallis one way ANOVA	H = 35.630	≤ 0.001
I3M GSL	0.06 ± 0.06	0.06 ± 0.03 c	0.18 ± 0.09 b	0.42 ± 0.12 a	Kruskal-Wallis one way ANOVA	H = 45.301	≤ 0.001
4MOI3M GSL	0.01 ± 0.01	0.02 ± 0.02 b	0.03 ± 0.02 b	0.12 ± 0.05 a	Kruskal-Wallis one way ANOVA	H = 39.109	≤ 0.001
1MOI3M GSL	0.25 ± 0.22	0.20 ± 0.09 b	0.17 ± 0.05 b	0.26 ± 0.09 a	Kruskal-Wallis one way ANOVA	H = 12.056	= 0.002
Total	1.18 ± 0.63	2.89 ± 0.81 b	4.51 ± 1.81 b	11.85 ± 2.14 a	Kruskal-Wallis one way ANOVA	H = 43.915	≤ 0.001

2OH3But, 2-hydroxy-3-butenyl; 2OH4Pent, 2-hydroxy-4-pentenyl; 5MSOP, 5-methylsulfinylpentyl; 2PE, 2-phenylethyl; I3M, indol-3-ylmethyl; 4OHI3M, 4-hydroxyindol-3-ylmethyl; 4MOI3M, 4-methoxyindol-3-ylmethyl; 1MOI3M, 1-methoxyindol-3-ylmethyl; different letters indicate significant differences between groups

Reference

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