Supporting Information

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SI Materials and Methods

Insects and Plants. Phyllotreta striolata adults were collected from fields of crucifer crops at AVRDC-The World Vegetable Center in Shanhua, Taiwan, and shipped to the Max Planck Institute for Chemical Ecology in Jena, Germany. The import authorization to Germany was obtained under Directive 2008/61/EC. Beetles used for volatile collections were maintained in a controlled environment chamber (25 °C, 75% relative humidity, 12-h/12-h light/dark period) and fed with organically grown Savoy cabbage, Brassica oleracea var. sabauda, which was purchased at a local grocery store. Adults used for feeding assays with different host plants were maintained on potted Arabidopsis thaliana myb28myb29 double-knockout (DKO) plants, which do not accumulate aliphatic glucosinolates (1), for at least 4 wk in a controlled environment chamber (24 °C, 65% relative humidity, 14-h/10-h light/dark period). This pretreatment was used to prevent uptake of aliphatic or aromatic glucosinolates and to provide equal conditions before starting the feeding experiments. Subsequent shipments of P. striolata adults were maintained on potted 3- to 5-wk-old Brassica juncea plants.

A. thaliana Columbia (Col-0) WT and *myb28myb29* DKO plants were cultivated in a growth chamber (21 °C, 50–60% relative humidity, 12-h/12-h light/dark period) until used for feeding of beetles. Seeds of *B. juncea* cv. "Bau Sin" and *Brassica rapa* var. *chinensis* cv. "Ching-Chiang" were purchased from Known-You Seed Co. Seeds of *Sinapis alba* were purchased from Treppens. Seeds of *B. oleracea* var. *italica* "Calabraise" were purchased from ISP International Seeds Processing GmbH; *B. oleracea* var. *italica* cv. "Valiant" seeds were purchased from Bruno Nebelung. *Brassica* spp. and *S. alba* were grown in a controlled environment chamber (24 °C, 65% relative humidity, 14-h/10-h light/dark period).

Feeding of *P. striolata* **on** *B. oleracea* **var**. *italica* **cv**. **Valiant**. Twenty *P. striolata* adults were allowed to feed on one 24-d-old *B. oleracea* var. *italica* cv. Valiant plant (three to four true leaves). After 24 h, beetles were removed and 200–300 mg of the youngest fully extended leaf was harvested for analysis of plant glucosinolate hydrolysis products. The remaining plant material was frozen in liquid nitrogen and freeze-dried for glucosinolate analysis. Undamaged plants of the same age served as controls. Four replicates were analyzed per treatment.

Volatile Collection and Analysis. The headspace of 30 male P. striolata feeding on the second or third youngest fully extended leaf of B. oleracea var. italica cv. Valiant (3-4 wk old, three to four true leaves), respectively, was collected for 24 h on an activated charcoal filter (1.5-mg closed-loop stripping analysis filter; Gränicher & Quartero). The plant material and insects were placed in a 100-mL silanized glass flask that was closed with a custom-made polyether ether ketone stopper. Airflow at 0.5 L/min was drawn through the flask by a rotary vane vacuum and pressure pump (G12/01 EB; Gardner) equipped with stainlesssteel adapters (IQSG-M54; Jenpneumatik). All tubings were made of Teflon (4-mm o.d., 2-mm i.d.). Incoming air was purified on an activated charcoal filter before entering the flask through a stainless steel mesh (SSD50; Jenpneumatik) to prevent adults from entering the tubing. Volatiles from mechanically damaged leaves of B. oleracea var. italica cv. Valiant (15 cm damaged using a pattern wheel) and empty flasks were collected under the same conditions and served as controls. Filters were eluted with 30 µL of hexane containing 10 ng/µL bromodecane (Sigma-Aldrich) as an internal standard.

A 1- μ L sample was injected in splitless mode in an Agilent Technologies 6890 N gas chromatograph equipped with an HP-5MS capillary column (30-m × 0.25-mm i.d., with 0.25- μ m film; J&W Scientific) coupled to a quadrupole mass spectrometer (5975B; Agilent Technologies). The carrier gas was helium at a constant flow of 1.1 mL/min. The inlet temperature was 250 °C. The oven program started at 40 °C for 3 min and increased at 10 °C/min to 280 °C. The temperature of the transfer line was 280 °C. MS conditions were electron impact mode (70 eV) and scan mode at 33–350 atomic mass units (amu). Isothiocyanates (ITCs) were identified by comparison of their mass spectra and retention times with those of authentic standards [allyl ITC, 95% purity (Sigma–Aldrich); 3-butenyl ITC, 95% purity (Tokyo Chemical Industry)].

Feeding Assay. To test the ability to accumulate different glucosinolate structures, adults were transferred to S. alba (17 d old, six to seven true leaves), B. juncea (20 d old, six to seven true leaves), B. rapa var. chinensis (20 d old, five to six true leaves), or A. thaliana Col-0 (40 d old, rosette stage). From each plant species except A. thaliana Col-0, three potted plants were placed in one mesh cage (MegaView Science Co.), together with 120 P. striolata adults. From A. thaliana Col-0, five potted plants were used for the feeding assay because plants were smaller. After 1, 3, and 7 d of feeding, groups of beetles were collected from the plants and kept on moistened tissue until the next day. By then, beetles should have an empty gut and were used for glucosinolate analysis. Thirty-five beetles that were collected before feeding on the test plants served as a control group. They were also kept in a box with moistened tissue until the next day. Beetles ready for glucosinolate analysis were collected in 1.5-mL reaction tubes (one to three adults per sample) and immediately frozen in liquid nitrogen. For each plant/day combination, 12 samples were collected. In addition, a group of beetles was fed for at least 4 wk on B. juncea to determine the maximum capacity of sequestered glucosinolates. Five samples (three beetles per sample) were collected for analysis of glucosinolates. Feeding assays were conducted under the same conditions as described for the P. striolata laboratory culture.

As a control, we tested whether beetles fed equally on the offered host plants *S. alba, B. juncea, B. rapa,* and *A. thaliana* Col-0 by comparing the remaining leaf area after feeding of five adult *P. striolata* on a leaf disk (20-mm diameter) for 24 h. The analysis of the quantified leaf areas (Adobe Photoshop CS5) revealed a weak significant difference between the four plant species (n = 27-29; ANOVA, F = 2.87, P = 0.04); however, there were no significant differences between the groups according to Tukey's honest significant difference post hoc test.

Glucosinolate Extraction and Analysis. The glucosinolate content of plants used in the feeding assay and in undamaged plants of the same stage was determined. Because feeding damage was observed all over the plant, the complete aboveground material was harvested, weighed, frozen in liquid nitrogen, and freeze-dried. From the corresponding dry weight of each sample, the dry matter content was calculated. Twenty milligrams of powdered sample was weighed into each well of a 96-well plate and extracted with 1 mL of 80% (vol/vol) methanol containing 50 μ M 4-hydroxybenzyl glucosinolate or allyl glucosinolate (for samples from *S. alba*) as an internal standard for 5 min on a horizontal shaker at room temperature. Afterward, plates were centrifuged for 10 min at 2,400 × g. DEAE-Sephadex A-25 (Sigma–Aldrich) columns were prepared in

96-well filter plates (Nunc). Columns were conditioned with 1 mL of ultrapure water and 1 mL of 80% (vol/vol) methanol before loading 600 µL of extract. Subsequently, columns were conditioned with 1 mL of 80% (vol/vol) methanol, followed by 2 mL of ultrapure water and 500 µL of 0.02 M MES buffer (pH 5.2). Thirty microliters of *Helix pomatia* sulfatase solution [prepared according to the method of Graser et al. (2)] was added to each sample and incubated at room temperature overnight. Desulfoglucosinolates were eluted from the DEAE-Sephadex A-25 column with 1 mL of ultrapure water into 96-deep well plates (Brand). Six replicates for undamaged plants and three replicates for feeding-damaged plants were analyzed, with the exception of five replicates of feedingdamaged A. thaliana Col-0 plants. Glucosinolates were extracted from P. striolata adults by grinding them directly in 200 µL of 80% (vol/vol) methanol containing the appropriate internal standard using a plastic pestle in a 1.5-mL tube. The volume of each sample was adjusted to 1 mL, and after thorough mixing, samples were centrifuged for 5 min at $4,800 \times g$. Extracts were transferred to DEAE-Sephadex A-25 columns prepared as described above. After the overnight incubation step with sulfatase, desulfoglucosinolates were eluted with 0.5 mL of ultrapure water.

Samples were analyzed by HPLC on an Agilent Technologies HP1100 Series instrument equipped with a photodiode array detector and a reversed phase column (NUCLEODUR Sphinx RP, 250×4.6 mm, 5-µm particle size; Macherey–Nagel) using an ultrapure water (solvent A) and acetonitrile (solvent B) gradient at a flow rate of 1 mL/min. The injection volume was 50 µL. The gradient was as follows: 1.5% (vol/vol) B (1 min), 1.5–5% (vol/vol) B (5 min), 5-7% (vol/vol) B (2 min), 7-21% (vol/vol) B (10 min), 21-29% (vol/vol) B (5 min), 29-100% (vol/vol) B (0.5 min), 100% (vol/vol) B (2.5 min), 100 to 1.5% (vol/vol) B (0.1 min), and 1.5% (vol/vol) B (4.9 min). The eluent was monitored by diode array detection between 190 and 360 nm. Desulfoglucosinolates were identified based on comparison of retention times and UV absorption spectra with those of known standards (3). The glucosinolate content in plants was calculated as nanomoles × milligrams of plant dry weight calculated from the peak areas at 229 nm relative to the peak area of the internal standard using relative response factors (4), and then converted to the corresponding concentration per milligram of plant fresh weight. For P. striolata adults, we determined the average fresh weight per beetle to be $1.12 \pm 0.14 \text{ mg} (n = 20)$ and used the approximate weight of 1 mg per adult to calculate the glucosinolate content in nanomoles × milligrams per beetle.

Data Analysis. The concentration of each glucosinolate in undamaged and feeding-damaged plants was compared using the Student t test or Mann–Whitney U test if data were not normally distributed. The glucosinolate profile of host plants (control and feeding-damaged) and beetles fed for 31 d or 49 d with A. thaliana myb28myb29 was determined, and the mean concentration for each quantified compound was calculated. To make glucosinolate contents in beetles comparable, the amounts are given as percentages. If beetles accumulated plant-derived glucosinolates, they were determined as the percentage relative to the mean concentration in feeding-damaged plants. If glucosinolates were not accumulated in the beetles (regardless whether the glucosinolate was present in the food plant or not), the relative percentage compared with the mean concentration in control beetles was calculated. To test whether plant glucosinolates were accumulated by the beetles, regressions were used. To compare the sequestration patterns of the main glucosinolates from the different plant species, and the sequestration pattern of a certain glucosinolate from different plant species, analyses of covariance were performed. If necessary, data were transformed to linearize data and/or to account for normality of the residuals and variance homogeneity. To test for differences in accumulation of different glucosinolates originating from one food plant, the method of generalized least squares was used. We applied the varIdent variance structure, which allows each glucosinolate to have its own variance (5). All analyses were done in R version 3.0.1 (6).

Glucosinolate Hydrolysis in *P. striolata* **Protein Extracts.** Adult *P. striolata* were starved for 1 d, killed using ethyl acetate vapor, and extracted in 50 mM MES buffer (pH 6.0) using a Dounce tissue homogenizer. After centrifugation and purification with 50 mg of DEAE-Sephadex A-25 to remove glucosinolates, the protein concentration of the supernatant was determined using the Bradford protein assay (Bio-Rad) according to the manufacturer's manual. Assays consisted of 20 μ g of protein and 1 mM glucosinolate [allyl glucosinolate, 4-methylsulfinylbutyl (4MSOB) glucosinolate, and 4-hydroxybenzyl glucosinolate, respectively] in 200 μ L of 50 mM MES (pH 6.0), and were incubated for 2 h at room temperature.

Analysis of Glucosinolate Hydrolysis Products. Harvested leaf material of B. oleracea var. italica cv. Valiant was crushed using a plastic pestle in 1 mL of ultrapure water and incubated for 10 min at room temperature. Benzonitrile (50 µL of a 1:10,000 dilution) was added as an internal standard, and samples were extracted subsequently using 2×2 mL of dichloromethane. Samples of *P. striolata* glucosinolate hydrolysis assays were extracted using $2 \times 750 \ \mu L$ of dichloromethane. The combined extracts were dried over a Na_2SO_4 column, reduced to 200–300 μL in a stream of nitrogen, and analyzed by GC coupled to a flame ionization detector (FID) using an Agilent Technologies 6890 N gas chromatograph equipped with a ZB5-MS capillary column $(30\text{-m} \times 0.25 \text{ mm-i.d.}, \text{ with } 0.25\text{-}\mu\text{m} \text{ film}; \text{Phenomenex})$. The carrier gas was hydrogen, and the detector temperature was 300 °C. One microliter of each sample was injected in splitless mode at 200 °C. The was oven program started at 40 °C for 3 min, was increased at 10 °C/min to 250 °C, and then at 60 °C/min to 300 °C, and was held for 3 min to clean the column. FID response factors of hydrolysis products relative to the internal standard benzonitrile were calculated by using the effective carbon number concept (7). Compounds were identified by GC-MS in comparison to authentic standards and published MS spectra (8). The GC oven program was identical to the GC-FID analysis.

Hydrolysis products of 4MSOB were directly analyzed using liquid chromatography (LC)-MS without extraction. Chromatographic analyses were carried out on 1100 series equipment (Agilent Technologies) coupled to an Esquire 6000 ESI-Ion Trap mass spectrometer (Bruker Daltonics) operated in positive ionization mode in the range of m/z 55–1,000, with a skimmer voltage of 38 eV, capillary exit voltage of 102.3 eV, capillary voltage of 4,000 V, nebulizer pressure of 35 psi, drying gas of 11 L/min, and gas temperature of 330 °C. Elution was accomplished using an EC 250/4.6 NUCLEODUR Sphinx RP column (250 mm \times 4.6 mm, 5-µm particle size; Macherey–Nagel) with a gradient of 0.2% formic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 1 mL/min at 25 °C as follows: 0-100% (vol/vol) B (25 min), 100% B (3 min), 100 to 0% B (0.1 min), and 0% B (4.9 min). Flow coming from the column was diverted at a ratio of 4:1 before reaching the electrospray ionization (ESI) unit.

Isolation of Intact 4MSOB. Intact glucosinolates were extracted from 50-g seeds of broccoli Calabraise using 350 mL of 80% (vol/vol) methanol following the protocol described by Badenes-Pérez et al. (9). To isolate 4MSOB, the aqueous extract was fractionated using an Agilent Technologies 1100 series instrument equipped with a SUPELCOSIL LC-18-DB column (250 mm \times 10 mm, 5-µm particle size; Supelco) connected to a fraction collector, by using a 0.05% TFA in ultrapure water (solvent A) acetonitrile (solvent B) gradient at a flow rate of 4 mL/min. The injection volume was 10 µL. The gradient was as follows: 3%

(vol/vol) B (6 min), 3-100% (vol/vol) B (0.1 min), 100% B (1.9 min), 100 to 0% B (0.1 min), and 0% B (3.9 min). The eluent was monitored with a UV detector at 229 nm.

Glucosinolate Sulfatase Assay. Crude protein extracts of P. striolata (prepared as described in Glucosinolate Hydrolysis in P. striolata Protein Extracts) were analyzed for glucosinolate sulfatase activity [method modified from that of Ratzka et al. (10)]. Twenty micrograms of protein in 50 µL of 50 mM MES (pH 6.0) was incubated with 50 µL of an aqueous solution of 5 mM glucosinolate (allyl glucosinolate, 4MSOB glucosinolate, indol-3ylmethyl glucosinolate, and 4-hydroxybenzyl glucosinolate, respectively) for 2 h at room temperature. As a positive control, the same amount of glucosinolate was incubated with 10 µL of sulfatase from H. pomatia [prepared according to Graser et al. (2)] and 40 µL of 50 mM MES buffer (pH 6.0). Assays were stopped by adding 500 µL of methanol, and intact glucosinolates were removed with 50 mg of DEAE-Sephadex A-25. The supernatant was dried with a stream of nitrogen and redissolved in 400 µL of ultrapure water. Desulfoglucosinolates were analyzed as described above. All tested glucosinolates were desulfated in assays performed with H. pomatia sulfatase; however, no conversion to desulfoglucosinolates could be detected upon incubation with P. striolata protein extracts.

RNA Isolation. Total RNA was extracted from *P. striolata* beetles using the RNeasy Lipid Tissue Kit (Qiagen), including on-column digestion of DNA using RNase-Free DNase (Qiagen) according to the instructions of the manufacturer. RNA integrity was verified using an Agilent Technologies 2100 Bioanalyzer with the RNA 6000 Nano Kit (Agilent Technologies), and RNA quantity was determined using a Nanodrop ND-1000 spectrophotometer. Five micrograms of total RNA was reverse-transcribed using the SuperScript First-Strand System for RT-PCR and Oligo (dT)₁₂₋₁₈ primer (Invitrogen). $Poly(A)^+$ mRNA was purified using the Poly (A)Purist MAG Kit (Invitrogen) and then used to prepare 5'- and 3'-RACE cDNA with the SMARTer RACE cDNA Amplification Kit (Clontech). For RNA sequencing (RNASeq), total RNA was extracted from both of the adult sexes and from male P. striolata 8 h after they were treated topically with juvenile hormone III (JHIII; purity $\geq 65\%$, 10 µg of JHIII in 0.5 µL of acetone per beetle; Sigma-Aldrich).

RNASeq and De Novo Transcriptome Assembly. RNASeq was performed with three P. striolata sample pools: control male and female adult beetles and JHIII-treated male beetles. RNASeq was performed on the HiSeq 2500 Sequencing System from Illumina (www.illumina.com/), using the paired-end 100-bp read technology. Illumina sequencing resulted in a total of 59, 69, and 67 Mio reads each for the female, male, and JHIII-treated male beetle samples, respectively. For the de novo assembly, 24 Mio reads of each of the three samples were used. Quality control, including filtering of high-quality reads based on the score value given in "fastq" files, and trimming of read length was done using CLC Genomics Workbench software v6.0.1 (www.clcbio.com). After these initial filtering steps, the de novo transcriptome assembly of the resulting 71 Mio total sequence reads was performed using CLC Genomics Workbench software by comparing an assembly with standard settings and two additional CLCbased assemblies with the following parameters: nucleotide mismatch cost = 2(3), insertion = deletion costs = 2(3), length fraction = 0.4(0.3), and similarity = 0.9(0.85). Scaffolding was selected, and conflicts among individual bases in all assemblies were resolved by voting for the base with the highest frequency. Contigs shorter than 250 bp were removed from the final analysis. The three assemblies were subsequently compared according to quality criteria, such as N50 contig size, total number of contigs, and the number of sequence reads not included in the

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contig assembly. For each assembly, the 100 largest contigs were manually inspected for chimeric sequences. The presumed optimal consensus transcriptome was selected among the three different assemblies based on the criteria of highest N50 contig size, lowest total number of contigs, and lowest number of chimeric sequences in the 100 largest contigs, which were found in the contig assembly with the following parameters: nucleotide mismatch cost = 2, insertion = deletion costs = 3, length fraction = 0.3, and similarity = 0.9.

Annotation. Annotation of the consensus transcriptome used BLAST searches conducted on a local server using the National Center for Biotechnology Information (NCBI) blastall program. Homology searches (BLASTx and BLASTn) of unique sequences and functional annotation by gene ontology (GO) terms (www.geneontology.org), InterPro terms (InterProScan; European Bioinformatics Institute), enzyme classification codes (ECs), and metabolic pathways (KEGG; Kyoto Encyclopedia of Genes and Genomes) were determined using the BLAST2GO PRO software suite v2.6.1 (www.blast2go.de) (11). Homology searches were performed remotely on the NCBI server through QBLAST against the NCBI nonredundant protein database using an E-value cutoff $\leq 10^{-1}$, with predicted polypeptides of a minimum length of 15 aa. ECs and KEGG metabolic pathway annotations were generated from the direct mapping of GO terms to their enzyme code equivalents. Finally, InterPro searches were performed remotely from BLAST2GO via the InterPro European Bioinformatics Institute web server.

Digital Gene Expression Analysis. Digital gene expression analysis was carried out using QSeq Software (DNAStar, Inc.) to remap the Illumina reads obtained from the control male and female adult beetles and JHIII-treated male beetles onto the reference backbone and then counting the sequences to estimate expression levels. For read mapping, we used the following parameters: n-mer length of 25; read assignment quality options required at least 25 bases (the amount of mappable sequence as a criterion for inclusion) and at least 90% of bases matching (minimum similarity fraction, defining the degree of preciseness required) within each read to be assigned to a specific contig; and 10 maximum hits for a read (reads matching a greater number of distinct places than this number are excluded). The n-mer repeat settings were automatically determined, and other settings were not changed. Biases in the sequence datasets and different transcript sizes were corrected using the reads per kilobase of transcript per million mapped reads algorithm to obtain correct estimates for relative expression levels.

Identification and Sequencing of Putative β -Glucosidases from *P. striolata*. Known myrosinases belong to the glycoside hydrolase family 1 (GH1) and are similar to β -glucosidases. We thus identified transcripts encoding putative β -glucosidases in the *P. striolata* transcriptome database based on homology to known β -glucosidases by using the Local Blast platform implemented in BioEdit (version 7.0.9.0). Full-length sequences of ORFs were obtained using 5'-and 3'-RACE PCR according to the manufacturer's protocols (Clontech), and were reamplified with specific primers (primer sequences are shown in Dataset S3) using the Advantage 2 Polymerase Mix (Clontech). PCR products were cloned into the TOPO TA 2.1 vector (Invitrogen) for sequencing. From three of a total of 28 GH1 β -glucosidase sequences, only partial sequences could be amplified. The manually curated cDNA sequences were submitted to the GenBank under accession numbers KF377828–KF377855.

Purification of a *P. striolata* Myrosinase. Beetles were frozen in liquid nitrogen and stored at -80 °C until used for protein extraction. Three hundred milligrams of starting material was ground in 1.4 mL of protein extraction buffer [20 mM Tris·HCl, 0.15 M NaCl, 5 mM EDTA (pH 8)] supplemented with protease inhibitors (cOmplete EDTA-free; Roche) using a Dounce tissue

homogenizer. The homogenate was centrifuged at 4 °C for 20 min at 16,000 \times g, and the supernatant was again ultracentrifuged at 4 °C for 30 min at 100,000 \times g. The crude beetle protein extract was tested for myrosinase activity (method described in Myrosinase Activity Assay) and then subjected to three subsequent chromatographic purification steps using an Akta FPLC system (GE Healthcare). First, size exclusion chromatography was performed using a Superdex 200 10/300 GL column (GE Healthcare) in Tris buffer [20 mM Tris HCl, 0.15 mM NaCl (pH 8)]. Fractions of 1 mL were collected from the elution with 2 column volumes (CV) at a flow rate of 0.5 mL/min. In the second purification step, glycoproteins were separated from nonglycosylated proteins using a 1-mL HiTrap Con-A 4B column (GE Healthcare). Active fractions were pooled and applied to a Zeba Spin Desalting column (Pierce) equilibrated with binding buffer [20 mM Tris-HCl, 0.5 M NaCl, 1 mM MnCl₂, 1 mM CaCl₂ (pH 7.4)]. The sample was applied to the Con-A column at a flow rate of 0.25 mL/min, the unbound protein was collected, and the column was washed with 10 CV of binding buffer at 1 mL/min. Glycoproteins were eluted with 4 CV of buffer containing 20 mM Tris-HCl, 0.5 M NaCl, and 0.5 M methyl-α-D-glucopyranoside (pH 7.4) at a flow rate of 0.25 mL/min. Excess sugar was removed from the eluate fraction by buffer exchange using a Zeba Spin Desalting column equilibrated with Tris buffer [20 mM Tris HCl (pH 8)]. Myrosinase activity was recovered in the eluate fraction, which was further purified on a 1-mL ResourceQ anion exchange column. After loading the sample, the column was washed with 10 CV of 20 mM Tris-HCl (pH 8). Bound protein was recovered in 1-mL fractions using a linear elution gradient from 0 to 1 M NaCl in 20 mM Tris HCl (pH 8) over 30 CV at a flow of 2 mL/min. Five hundred microliters from the fraction containing the highest myrosinase activity was used for protein precipitation with 10% (vol/vol) TCA and 0.02% deoxycholate. The pellet was dissolved and boiled in 20 μ L of 1 \times XT sample buffer (Bio-Rad) and loaded on a Criterion 12.5% (wt/vol) polyacrylamide SDS/PAGE gel. The gel was run for 80 min at 120 V and afterward stained with colloidal Coomassie blue (Roti-Blue, Carl Roth).

In-Gel Tryptic Digestion and Peptide Extraction. Protein bands between 25 and 100 kDa were excised from the gel, cut in small pieces, washed several times with 25 mM NH₄HCO₃, and destained with 25 mM NH₄HCO₃/50% (vol/vol) acetonitrile. Disulfide bonds were reduced with 10 mM DTT at 50 °C for 1 h and alkylated with 55 mM iodoacetamide at room temperature in the dark for 45 min. Following tryptic digestion in a 0.5 μ M solution of porcine trypsin (Promega) in 25 mM NH₄HCO₃ overnight at 37 °C, the peptides were extracted from the gel pieces using 75% (vol/vol) acetonitrile/5% (vol/vol) formic acid and dried down in a vacuum centrifuge (12). For MS analysis, samples were dissolved in 10 μ L of 0.1% formic acid.

Nano-Ultraperformance LC-Tandem MS Analysis. The peptide mixtures (1–8 μ L) were initially concentrated and desalted on a Symmetry C18 trap-column (20 × 0.18 mm, 5- μ m particle size; Waters) using 0.1% formic acid as a mobile phase at a flow rate of 15 μ L/ min. Peptides were then separated on a nanoAcquity C18 analytical column (200 mm × 75 μ m i.d., C18 BEH 130 material, 1.7- μ m particle size; Waters) using a 0.1% formic acid in ultrapure water (solvent A) acetonitrile (solvent B) gradient at a flow rate of 350 nL/min. The applied LC gradient was as follows: 1–30% (vol/vol) B (13 min), 30–50% (vol/vol) B (5 min), 50–95% (vol/vol) B (5 min), 95% (vol/vol) B (4 min), and 95 to 1% B (1 min). The analytical column was reequilibrated for 9 min before the next injection.

The eluted peptides were online-transferred via a Nano-LockSpray ion source into a Synapt high-definition tandem mass spectrometer (Waters). The source temperature was set to 80 $^{\circ}$ C, cone gas flow was set to 30 L/h, and the nanoelectrospray voltage was 3.2 kV. For all measurements, the mass spectrometer was

operated in V-mode with a resolution power of at least 10,000 FWHM. All analyses were performed in positive ESI mode. The lock-mass calibrant standard, human Glu-Fibrinopeptide B [650 fmol/mL in 0.1% formic acid/acetonitrile (vol/vol, 1:1); Sigma–Aldrich], was infused into the NanoLockSpray electrospray source at a flow rate of 500 nL/min through the reference sprayer every 30 s to compensate for mass shifts in MS and tandem MS (MS/MS) fragmentation mode.

LC-MS data were collected using MassLynx v4.1 software under data-dependent (DDA) and data-independent/LC-elevated energy (MS^E) acquisition. For DDA, the acquisition cycle consisted of a survey scan covering the range of m/z 400–1,700 Da, followed by MS/MS fragmentation of the four most intense precursor ions collected at 1-s intervals in the range of 50–1,700 m/z. Dynamic exclusion was applied to minimize multiple fragmentations for the same precursor ions. For LC-MS^E analyses, full-scan LC-MS data were collected using an alternating mode of acquisition: low-energy (MS) and MS^E modes at 1.5-s intervals with a 0.2-s interscan delay in the range of 300–1,900 and 50–1,700 m/z, respectively. The collision energy of the low-energy MS mode and MS^E mode were set to 4 eV and 15–40 eV energy ramping, respectively.

Data Processing and Protein Identification. ProteinLynx Global Server (PLGS) version 2.5.2 (Waters) was used for processing of raw files and for database searching. DDA raw files were initially baselinesubtracted, smoothed, deisotoped, and lock-mass-corrected, and pkl files were generated. Processed MS/MS spectra (pkl files) were searched against the NCBInr database (updated on December 5, 2012, containing 21,786,050 sequence entries) combined with the P. striolata protein subdatabase (containing 282,360 entries, constructed from the inhouse-created EST database by its translation from all six reading frames) using MASCOT v2.3 software installed on a local server. Trypsin was set as the primary digest reagent, and one missed trypsin cleavage site was allowed. Mass tolerances for precursor and fragment ions were 15 ppm and 0.03 Da, respectively. A fixed modification of carbamidomethyl-cysteine was specified, and oxidation-methionine was set as a variable modification. Proteins matched by at least three peptides with ion scores above 30 or by one peptide with a protein score higher than 55 were considered as correct assignments.

The continuum LC-MS^E data were lock-mass-corrected, smoothed, background-subtracted, centered, deisotoped, and charge state-reduced using the PLGS software. Product ion spectra were generated using the following thresholds for low/high-energy scan ions and peptide intensity: 150, 30, and 750 counts, respectively. Time-based alignment of precursor and fragment ions was done using the Ion Accounting Algorithm as described by Li et al. (13). Processed data were searched against the constructed P. striolata transcriptome database (as described above) combined with the UniProt database (downloaded on July 27, 2011, from www.uniprot.org/). Database searching was restricted to tryptic peptides with a fixed carbamidomethyl modification for cysteine residues, along with variable oxidation of methionine. Further, default searching parameters specifying mass measurement accuracy were used, with a minimum of five product ion matches per peptide, a minimum of seven product ion matches per protein, a minimum of two peptide matches, and a maximum of one missed tryptic cleavage site. The maximum falsediscovery rate (FDR) was set to 2%, and all peptides matched under the 2% FDR were considered as correct assignments.

According to this identification method, peptides corresponding to the same transcript were often identified in several proteomic samples (Dataset S2), which may be partly explained by protein degradation during the purification process. For example, beetle myrosinase peptides were found in 14 of 16 samples. However, only three transcripts were assigned to the dominant protein band in the active fraction (Fig. S4, sample 5): GH1-2, GH1-4, and the myrosinase. It is thus likely that the identified beetle myrosinase principally corresponds to this proteomic sample. Cloning and Expression of Myrosinase Candidate Genes. Database searches of the obtained proteomic data against the P. striolata transcriptome returned six transcripts encoding putative β-glucosidases. These transcipts were amplified, including the predicted Nterminal signal peptide from cDNAs, by PCR (Advantage 2 Polymerase Mix), using gene-specific primers (primer sequences are shown in Dataset S3) and ligated into the pIB/V5-His TOPO TA expression vector (Invitrogen). The forward primer was designed, including a 5'-Kozak sequence; the reverse primer lacked the native stop codon for C-terminal fusion to the vector-encoded V5 epitope and His-tag. The correct orientation and sequence of the insert were confirmed by sequencing; at least two clones from each construct were used for transfection. Confluent Sf9 cells cultivated in Sf-900 II SF medium (Invitrogen) were diluted 1:4, and 500 µL were transferred to each well of a 24-well plate. After overnight incubation at 27 °C, cells were transfected using FuGENE HD Transfection Reagent (Promega) according to the manufacturer's protocol.

To obtain a stable transfected cell line expressing the beetle myrosinase, the corresponding plasmid was used to transfect a 70% confluent monolayer of *Sf9* cells prepared in a 60-mm² Petri dish. Upon confluence, selection for transfected *Sf9* cells was performed with blasticidin at 80 μ g/mL. In the stable transfected line, selection pressure was maintained using 10 μ g/mL blasticidin.

Western Blots. Cell culture medium was harvested after incubation for 72 h at 27 °C. From cell cultures containing GH1-10, cytosolic proteins were extracted using Insect Pop Culture reagent (Merck Millipore) supplemented with benzonase (Novagen) according to the manufacturer's instructions. The cell pellet was additionally extracted for 1 h at 4 °C with constant gentle agitation using 1% Triton X-100 in PBS buffer supplemented with protease inhibitors. After centrifugation, supernatant and remaining cell pellets were stored separately for later analysis. The crude extracts were used for Western blot analysis using goat anti-V5 antibody HRP-conjugated (1:20,000; Bethyl Antibodies).

Myrosinase Activity Assay. Myrosinase activity in the crude beetle extracts, fractions obtained from the protein purification procedure, and samples of Sf9-expressed GH1 enzymes from P. striolata was analyzed by qualitative detection of the volatile glucosinolate hydrolysis product allyl ITC using headspace solidphase microextraction (SPME) coupled with GC-MS. Assays containing 10 µL of sample and 50 µL of 40 mM sodium acetate buffer (pH 6) with 10 mM allyl glucosinolate as a substrate were performed in 1-mL glass vials. Control assays were performed without substrate and without protein extract (only buffer). After penetrating the polytetrafluoroethylene-lined silicone septum of the cap with the SPME fiber holder, the SPME fiber coated with 100 µm of polydimethylsiloxane (Supelco) was exposed to the headspace in the vial for 10 min at room temperature. Afterward, the SPME fiber was directly inserted into the inlet of an Agilent Technologies 6890 N gas chromatograph equipped with an HP-5MS capillary column (30-m \times 0.25-mm i.d., 0.25- μ m film; J&W Scientific) coupled to an Agilent Technologies 5975B quadrupole mass spectrometer. The carrier gas was helium at a constant flow rate (1.1 mL/min). The inlet temperature was set to 250 °C for simultaneous conditioning of the SPME fiber. The oven program was started at 40 °C for 1 min, was increased at 20 °C/min to 130 °C, and then with 50 °C/min to 240 °C, and was held for 2 min. MS conditions were electron impact mode (70 eV) and scan mode (33-350 amu). Allyl ITC was identified based on its mass spectrum and comparison with an authentic standard (95% purity; Sigma-Aldrich). Thermal conversion of allyl ITC in the hot GC inlet and MS transfer line is responsible for the detection of allyl thiocyanate in these samples (14). This compound was not detectable when the inlet temperature was set to 100 °C.

Partial Purification and Characterization of Recombinant P. striolata Myrosinase. HisPur Cobalt Resin (Pierce) was used to concentrate and purify recombinant beetle myrosinase from Sf9 cell culture medium via the His-tag using a gravity-flow column according to the manufacturer's protocol for native conditions. The purity was checked with SDS/PAGE, followed by colloidal Coomassie staining; however, the result was unsatisfactory, probably due to the very low expression level in the cell culture. The sample was dialyzed against storage buffer [20 mM Tris·HCl, 0.15 M NaCl (pH 8)], and the total protein concentration was determined using the Bradford protein assay (Bio-Rad) according to the manufacturer's manual. For determination of the optimal pH, myrosinase activity assays were performed using citrate-phosphate buffers covering pH 3-7.6 and 2 mM allyl glucosinolate (Carl Roth) as a substrate. After incubation for 20 min at room temperature, the reaction was heat-inactivated and released glucose was quantified using the Glucose (HK) Assay Kit (Sigma-Aldrich).

To test the substrate specificity of the beetle myrosinase, activity toward the following substrates was analyzed: allyl glucosinolate (Roth); 3-butenyl glucosinolate, 4-pentenyl glucosinolate, and indol-3-ylmethyl glucosinolate (Phytoplan); 4MSOB glucosinolate (isolated as described above); and 4-hydroxybenzyl glucosinolate (isolated from S. alba seeds). In addition, the general substrates for β-glucosidases, 4-methylumbelliferyl-β-D-glucopyranoside (99% purity; Acros Organics) and 4-nitrophenyl-B-D-glucopyranoside (>98% purity; Sigma-Aldrich), were tested. Assays in were performed 96well plates using the Amplite Glucose Quantitation Kit (AAT Bioquest) and consisted of 25 μ L of protein sample (20 ng of total protein), 25 µL of substrate at 5 mM [prepared in citrate-phosphate buffer (pH 6.5), 1.25 mM final concentration], and 50 µL of assay reagent [prepared with citrate-phosphate buffer (pH 6.5) instead of the supplied buffer]. Assays without substrate served as background controls. Three replicates were measured for each substrate and the background control. Fluorescence intensity (excitation/emission = 540 nm/590 nm) was monitored every 90 s for 60 min using a Tecan Infinite 200 Reader. The mean of the background control was subtracted from each value, and released amounts of glucose were calculated from a glucose standard curve for each time point. Myrosinase activity was calculated from the linear range of the monitored reaction (between 15 and 25 min) and is given as nanomoles of glucose per milligram of total protein per minute.

Phylogenetic Analysis. Protein sequences from the aphid myrosinase and putative β -glucosidases from the pea aphid, Acyrthosiphon pisum (Database: aphidbase_RefSeq_peptides), as well as from two Coleoptera, Tribolium castaneum and Dendroctonus ponderosae, and other insects, were retrieved from publicly available databases and aligned with the deduced protein sequences from *P. striolata* using the multiple alignment program for amino acid or nucleotide sequences (MAFFT) with default settings (version 7, http://mafft.cbrc.jp/alignment/server/). Poorly aligned regions were eliminated from the alignment using GBlocks (version 0.91b) with parameters set as follows: minimum length of 5 amino acids for a block after cleaning, minimum of 36 sequences for a conserved position and for a flank position, maximum of eight contiguous nonconserved positions, and half of gap positions allowed. The maximum likelihood tree was reconstructed in MEGA 5.05 using the WAG + G + I model with pairwise deletion of gaps, and was tested with 1,000 bootstrap replicates. A Bayesian phylogenetic analysis consisting of four Markov chains with 10,000,000 generations that were sampled every 100 generations was carried out in MrBayes v.3.2.1. The first 25% of saved trees were discarded.

The phylogenetic analysis of the GH1 β -glucosidase family in *P. striolata* revealed several transcripts closely related to the beetle myrosinase. These transcripts were cloned and expressed as described above and tested for myrosinase activity. GH1-10 was additionally expressed without the N-terminal signal peptide for intracellular expression; however, in both cases, the ex-

pressed protein was mainly detected in the cell debris fraction using Western blotting.

Analysis of Predicted Amino Acid Sequences of *P. striolata* GH1 β -Glucosidases. The molecular mass, isoelectric point, and presence of a signal peptide of putative *P. striolata* β -glucosidases and the myrosinase were predicted using the ProtParam tool (15), and SignalP 4.1 (16), respectively. The number of putative *N*glycosylation sites was determined using NetNGlyc 1.0, which was provided at CBS Prediction Servers.

SI Results

To determine the profile of glucosinolate hydrolysis products in *P. striolata*, we analyzed *P. striolata* protein extracts that were

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incubated with allyl-glucosinolate, 4MSOB glucosinolate, and 4-hydroxybenzyl glucosinolate, respectively, and subsequently extracted using dichloromethane, with GC-FID and GC-MS. Only allyl ITC could be detected as a hydrolysis product of allyl glucosinolate, whereas hydrolysis products of 4MSOB glucosinolate and 4-hydroxybenzyl glucosinolate were below the detection limit with this method. Assays performed with 4MSOB glucosinolate as a substrate were additionally directly analyzed using LC-MS (Fig. S3). The extracted ion chromatograms for 4MSOB-ITC and 4MSOB-nitrile showed that the ITC was the dominant product. A small amount of nitrile was detected, but some 4MSOB-nitrile was also present as a contaminant in the substrate.

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Fig. S1. Glucosinolate profile of *B. oleracea* var. *italica* cv. Valiant and headspace volatiles of mechanically and *P. striolata*-damaged leaves. (A) Glucosinolate concentration in undamaged and *P. striolata*-damaged 24-d-old plants (20 adults per plant for 24 h; n = 4, +SD). I3M, indol-3-ylmethyl; 1MOI3M, 1-methoxyindol-3-ylmethyl; 4MSOB, 4-methylsulfinylpropyl; 3MSOP, 3-methylsulfinylpropyl; 4MOI3M, 4-methoxyindol-3-ylmethyl; 4OHI3M, 4-hydroxyindol-3-ylmethyl. (*B*) GC-MS analyses (total ion chromatograms) of volatile collections from broccoli leaves with 30 feeding *P. striolata* for 24 h (*Upper*) and mechanically damaged leaves (*Lower*, inverted).



Fig. 52. Change of the glucosinolate profile in *P. striolata* feeding on *B. juncea* (n = 12), *B. rapa* (n = 11-12), *A. thaliana* Col-0 (n = 11-12), and *S. alba* (n = 7-12) for 1, 3, and 7 d [linear regression of transformed data (Dataset S1); n.s., P > 0.05; *P < 0.05; *P < 0.05; *P < 0.01; **P < 0.01]. All values are given as mean \pm SEM. (*A*, *D*, *G*, and *J*) Accumulation of glucosinolates from the respective host plant calculated as percentage relative to the concentration in induced plants (100%) over time. (*B*, *E*, *H*, and *K*) Host plant glucosinolates that were not accumulated over time; changes are calculated relative to the concentration in the *P. striolata* control group (100%). (*C*, *F*, *I*, and *L*) Changes of previously sequestered glucosinolates relative to the concentration in the beetle control group are depicted. 3But, 3-butenyl; 4MSO3But, 4-methylsulfinyl-3-butenyl; 7MSOH, 7-methylsulfinylheptyl; 8MSOO, 8-methylsulfinyloctyl; 5MSOP, 5-methylsulfinylpentyl; 4MTB, 4-methylthio-3-butenyl; 4OHBenzyl, 4-hydroxybenzyl; 2OH3But, 2-hydroxy-3-butenyl; 4Pent, 4-pentenyl.



Fig. S3. LC-MS (ion trap)-extracted ion chromatograms of 4MSOB-nitrile (m/z = 146) and 4MSOB-ITC (m/z = 178) in positive ionization mode. Control of protein extract without substrate showed no signals for either mass and is not shown. (A) Adults were not frozen and extracted using buffer pH 6 without EDTA (recommended for measuring activity of specifier proteins). (B) Control without protein shows traces of 4MSOB-nitrile as a contamination in the substrate.



Fig. 54. One-dimensional SDS/PAGE gel of final fraction obtained from activity-guided protein purification (corresponding to 500 μL of the 1-mL fraction). Numbers indicate samples excised from the gel for in-gel tryptic digestion and peptide extraction for proteomic analyses.



Fig. S5. Western blot analysis of *Sf9*-expressed myrosinase candidates. Control A corresponds to culture medium. Control B corresponds to the remaining cell pellet from *Sf9* cell culture treated only with the transfection reagent. GH1-6, GH1-2, GH1-9, GH1-7, GH1-4, and the myrosinase (Myr) were positively identified in the active fraction with LC-MS^E; GH1-8, GH1-3, GH1-1, and GH1-10 are phylogenetically related to the beetle myrosinase. GH1-10 was mainly detected in the cell pellet.

	\diamond	
<i>Pst</i> Myr <i>Bbr</i> Myr <i>Sal</i> Myr	<u>MQQTIAFVLVLLQFAFNADG</u> ALKNNGQFPRNFLFGAATASYQIEGAWNEDGKGQSTWDEFTHRIFSPV-TNNDTGDIACDHYHRYKEDIK 	89 66 84
<i>Pst</i> Myr <i>Bbr</i> Myr <i>Sal</i> Myr	<pre></pre>	178 154 174
<i>Pst</i> Myr <i>Bbr</i> Myr <i>Sal</i> Myr	©▼ L-PKVGYWSTINEPRVHCLRSYGDGKHAPGIAESGIADYQCSYVILKAHAKAYRMYKKEF-PHYKAPFGIVIDCQWYVP FGDRVKWWITENEPIAVC-KGYSIKAYAPNLNLKTTGHYLAGHTQLIAHGKAYRLYEEMFKPTQNGKISISISGVFFMP FGDSVKYWLTINQLYSVPTRGYGSALDAPGRCSPTVDPSCYAGNSSTEPYIVAHHQLLAHAKVVDLYRKNY-THQGGKIGPTMITRWFLP	255 232 263
<i>Pst</i> Myr <i>Bbr</i> Myr <i>Sal</i> Myr	<pre></pre>	344 321 338
<i>Pst</i> Myr <i>Bbr</i> Myr <i>Sal</i> Myr	DEINYKHDVRVNLFNDPSW-KISVLGWAI-CPFGVRELLKHLKEOYGNEPIIFAEIGVADDGTSLKD PNFNPDASYVTSVDEAWLKPNETPYIIPVPEGLRKLLWLKNEYGNPQLLITENGYGDDGQLDD PNPVNATNHTAMMDAGAKLTYINASGHYIGPLFESDGGDGSSNIYYYPKGIYSVMDYFKNKYYNPLIYVTENGISTPGSENRKESMLD	409 385 426
<i>Pst</i> Myr <i>Bbr</i> Myr <i>Sal</i> Mvr	¢ DIRTEYYQGYFCYILEAMQIDDVNVVAIIPWSLMDNLEWAKCYIYHFGLIGIDFYNDPTLTRRLKDSTYFYKNVTTTKRINCDNVK 495 FEKISYLKNYLNATLQAMYEDKCNVIGYTVWSLLDNFEWFYCYSIHFGLVKIDF-NDPQRTRTKRESYTYFKNVVSTGKP 464 YTRIDYLCSHLCFLNKVIKEKDVNVKGYLAWALGDNYEFNNCFTVRFGLSYINW-NNVT-DRDLKKSGOWYOKFISP 501	

Fig. S6. Amino acid sequence alignment of myrosinases from *P. striolata (Pst)*, *B. brassicae (Bbr*, UniProt accession no. Q95X01.1), and *S. alba (Sal*, from Protein Data Bank accession no. 1MYR_A). The predicted signal peptide for the secretory pathway of the beetle myrosinase is doubly underlined, and the predicted *N*-glycosylation sites are shown in bold. Catalytically active residues are marked with a black triangle (∇), and residues involved in glucose binding are marked with a lozenge (\Diamond). The first catalytic glutamate residue is substituted by a glutamine in all plant myrosinases. The aglycone recognition site of the plant myrosinase is marked with black circles (\bullet) below the sequence. Overall identical residues are printed white on a black background, and similar residues are highlighted with a gray background.

THAT OBTHOSE	SERIKNNGOPPRNFLEGAATASKOIPGAWNEDCKCOST	WDEFT RIRSPUTNNDTGDIACDHYHRYKDDIKMAAD WEDOKYRSISWPRULPNCYA-DTINEKCMOFYKDW 108
GH1-1	SHLKNNGIFPKNFIFGAATSAYQIEGAWNADGKGOSI	WDEFVHRSPSPIANNETCDVACDSYHRWKEDVHLVSNICMOYYRFSISWSRLFPSGYANEKINEAGLKYYKKL 110
GH1-8	SHLKNNGVFPKKFIFGAATAAYQIEGAWNEDGKGQSV	WDEFVHRSPSPIANNETGDVACDSYHRWKEDLHUSNLCMQYYRFSIAWTRLFPDGYVGKKINQAGLIYYKKL 110
GH1-3	LKNNGRFPNDFLFGVSTASYQIEGAWLEDGKGLSV	WDDFVHRNESLVVNNDTGDVACDSYHRFREDVHETACLGVQLYRFSISWARLFPDGYV-DKINQKGLRYYKNL 107
GH1-10	DDVLKNNGOFPRNFMFGAASSAYOIEGAWNEDGKGLST	WDHFVRRTPSPVVDNTTGDWACDSYRRWKEDVKUVADLGVOSYRFSISWPRILPSGYD-KHWNEKGLEFYKOL 110
GH1-21	EOTPKKHNGHEPKHELLGAGTSAYOWEGAWNLDGKGPSI	WDEFAHKHPERIJDHSNADMTIDWYHRYKED LALASSIC KLERFSESWPRIFPNGYN-TTINPHGYKEYKNF 111
GH1-2	EKINTKRFPSDFLFGTATAAYOIEGAWNEDGKGVNI	WDTTCHENPSPIANNATADHACDSYHKYKEDHALLKDMGVDVYRFSISWSRILPLGYD-NVKNOLGLDYYKNF 108
GH1-16	SSVNNKKEPDSELUGTATAAYOTEGAWNEDGKGENT	WORTCH SDPTR TENNENAD JACDSYH WYKED VAL LKDLGVSVYRESTSWSRTLPTGYL-NETNOLGTNYYKNT 108
GH1-13	YELNRKKEPDEERIGVATSAYOTEGCWNEDGKGENT	WDRAVESSPSPTEDOTTADUACDSYHWYKEDVCUAKDLGVDHYRESISWSRTLPTGFA-NHINFAGWNYYKNU 108
GH1-9	DDPVINNKKEPLDELEGTATSAYOIEGAWNLDGKGENI	WDRATHTNRSFTKNGDSGDVACDSYHTYKEDVONLOYLGVNHYRESISWSRULPDGLA-HRVNOAGVRYYRNU 110
GH1-20	EEHINNTPEPKDELEGTATSAYOWEGAWNVDGKGVNN	WINKETH TNRSPITKENDTA DVA CDSYHRYKENVAL LKYLEVOFYRESTSWIRTLENGTO-GKINOOGVOYYKNI 109
GH1-17		MINIT THEN PARTICIPATION OF A DESCRIPTION AND A DESCRIPTION OF A DESCRIPTI
CH1_10		
CH1_14		
GH1-14 CW1-15		
CH1_19		
GH1-15 CH1-25		
GHI-ZJ		
GHI-0		
GHI-/		WDRFAHKHEDKFYDRSTPDTACDSYRNYEDTADVKARGEDHYRLSTAMSRVLPTGTT-DKVNKAGVEYTKNV 109
GHI-23	DDUNTRY PRICE OF A DOUBLE OF A	WDRFARARPEKIADRSTPDVACDSYRKYKEDIATIKANGEDRYRESTAWSRVLPTGIT-DKVNAGVEYYKNV 108
GHI-4		WDTFTHNHBELISDRSNADVTCDSYHRYKEDVTDLERLGGVNIYRFSISWSRILPTGLP-DEINENGIOYYRNL 110
GH1-24		WDRSSHATHYRF-DDADADWACDSYHRYREDVARDARLGTHYRFSISWARTYPTGFN-NRUNTREVULYYONL 107
GH1-22		WDRWSHERRAGDADAGDADTASDSYHRYREDTOTARELGTHYMFSISWARTFPSGFN-NSFSTQAQFHYOSL 110
GH1-5	MAEKKNDSKFPDDFIFGVSTSAYQIEGSTDVDGRGENN	NDFYIRTNPEKF-NHETALIACDSYSKWRGDIEDIRSLGVNTYRFSISWNRILPTGYA-NRINOKAIKHYSDI 109
GH1-12	MIKSIFQATVILLCTKNSLEKTDFEKHRFPADFSFGVASSAYQIEGGYNEDGKGESI	EDHYTKTREKKFFNETNGNVACDSYHKWRTDVRDLADLGVDFYRFSISWSRILPOGES-RPINGDGLRYYNDL 129
GHI-II	MLWNIESFSKPHDPLMPIPLPEIHGIFPIEFSFGISGGAFEHEGAWLEDNKGESI	WDHWVHENDNVTVDNGNGVVSADSYNHVNDDVDDLANLGVNHFVFSISWGRIDPAGEGDVNMLGINHYKKL 126
GH1-26		YXAVVDGSNGUIASUAMHHIEDUVKILDHUNYRHYKESISWSRIDPNGIGDINREGIKHYQKU 63
GH1-27		1
4	e VDEIVKYGMVPVCTLFHWDLPLKLYENGIDWRNEKIIDIFVAYSRLMIQNL-PKV	GY <mark>WSTINEPRVHC</mark> LRS <mark>YGDGKHAP</mark> GIAE-SGIADYCCSYVIIKAHAKAYRMYKKEF-PHYKAPFGIVIDCQW 232
GH1-1 GH1-8 GH1-3 GH1-10 GH1-21 GH1-2 GH1-12 GH1-12 GH1-13 GH1-20 GH1-17 GH1-19 GH1-14 GH1-15 GH1-18 GH1-25 GH1-6 GH1-7 GH1-23 GH1-4 GH1-24 GH1-22 GH1-5	96 VDETVKYGMVENCTLEHNDLELKYYENGTLENNEKTIDIE VAYSELINGNL-PKV TCSVIKRGILEVVTLEHNDLELKYYDGEISMTNDSIIDIEVAYSERVIKEP-PEVGA 105 VVKLGIMEVVTLEHNDLELTFODGLSMTNDSIIDIETCYAKVIENG-PEV UCSIIKKONENGTEHNDLELTFODGLSMTNDAIDIETCYAKVIENG-PEV VCSIIKKONENGTEHNDLELTFODGLSMTNPALIDIETCYAKVVIENG-PEV RELKYDNIEUTYHNDLELOYNDELINNIPGIVNIEVYARFVIKNI-PEV IRSLKANNEEUTYIHNDLEUCYNDGIINNIPGIVNIEVYARFVIKNI-PEV ISSLKANNEEUTYIHNDLEUCYDDIEGENERFYVERSDEXAEUCYARVVOIDNGDDV ISSLKANNEEUTYIHNDLEOFDDI-GGIAGENEYVERSDEXAEUCYARVVOIDNGDDV IASJEKANNEEUTYIHNDLEOFDDI-GGIAGENEYVERSDEXAEUCGUEGOU IASJEKANNEEUTYIHNDLEOFDDI-GGIAGENEYVERSDEXAEUCGUEGOU IKELKANNEEUTYIHNDLEOFDDI-GGIAGENEYVERSDEXAEUCGUEGOU IKELKANNEEUTYIHNDLEOFDDI-GGIAGENEYVERSDEXAEUCGUEGOU IKELKANNEEUTYIHNDEOFDDI-GGIAGENEYVERSDEXAEUCGUEGOU IKELKANNEEUTYIHNDLEOTDOL-GGENDEYVERSDEXAEUCGUEGOU IKELKANNEEUTYIHNDEOFDOL-GGIAGENEYVERSDEXAEUCGUEGOU IKELKNNEEPTYIHNDEOFDOL-GGIAGENEYVERSDEXAEUCGUEGOU IKELKNNEEPTYIHNDEOFDOL-GGIAGENEYVERSDEXAEUCGUEGOU IKELKNNEEPTYIHNDEOFDOL-GGIAGENTAEUVHYSRYAEUCGUEGOU IKELKNNEEPTYIHNDEOFDOL-GGIAGENTAEUVHYSRYAEUCGUEGOU IKELKNNEEPTYIHNDEOFDOL-GGIAGENTYKIEGENETUNGEGEVEGUEGOU IKELKNNEEPTYIHNDEOFDOL-GGUEGENEYVERSDEXTAEUCGUEGOU IKELKNNEEPTYIHNDEOFDOL-GGUEGENEYVENEEPTYNEEGENEGENE- IKELKNNEEPTYIHNDEOFDOL-GGUEGENEYVENEEPTYNEEGENEGENE- ISELKINNEEPTYIHNDEOFDOL-GGUEGENEN FERTERAADUKENUTYHNDEOFDOL-GGUEGENEN FKOLKANDUKENUTYHNDEOFDOL-GGUENESIVVEGUXAEUTYKENEEPTYNEE FKOLKANDUKENUTYHNDEOFDOLOGUNAEUNESIVVEGUXAEUTYKENEEPTYNEED FKOLKANDUKENUTYHNDEOFDOLOGUNAEUNESIVVEGUXAEUTYKENEEPTYN IDEULKINNTERATIYHNDEOFDOLOGUNAEUNESIVVEGUXAEUTYKENEEDDA VNELKHNTIPAATIYHNDEOFDOLOGUNAEUNESIVVEGUXAEUTYKENEEDDA VNELKHNTIPAATIYHNDEOFDOLOGUNAEUNESIVVEGUXAEUTYKENEEDDA VNELKHNTIPAATIYHNDLEOFDOLOGUNAEUNESIVVEGUXAEUTYKENEEDDA IDEUKANDUKEUTYHNDLEOFDOLOGUNAEUNESIVVEGUXAEUTYKENEEDDA IDEUKANDUKEUTYHNDLEOFDOLOGUNAEUNESIVVEGUXAEUTYKENEEDDA- VNELKHNTIPAATIYHNDLEOFDOLOGUNAEUNESIVVEGUXAEUTYKENEEDDA IDEUKANDUKEUTYHNDLEOFDOLOGUNAEUNESIVVEGUXAEUTYKENEEDDA NEULKHNTIPAATIYHNDLESIDENEESIE	CYNGINERRYHCLRSYGGEKHARGIAE-SGILDCOGYVIIRAAAXYRYKKEE -PHYKAPFGIVIICOC 223 ITGKMALINERRHGRNSYGGEVILAGGIKE-SGILDCOGYVIIRAAAYRYKKEE -PHYKAPFGIVICOC 234 -SIMITVNERIYGYSGEULAGGIKE-SGILDCOGYVIIRAAAYRYKKEE -PHYKAPFGIVUCCO 234 -SIMITVNERIYGYSGEVILAGGIKE-SGILDCOGYVIIRAAAYRYKKEE -PHYKAPFGIVUCCO 234 -GIMVSINERYYGYSGEVILAGGIKE-SGILDCOGYVIIRAAAYRWYKKEE -PHYKAPFGIVUCCO 234 -GIMVSINERYYGYSGEVILAGGIKE-SGILDCOGYVIIRAAAYRWYKKEE -PHYKAPFGIVUCCO 234 -GIMVSINEPHTICHLGYSIGULAFGIKG-DGKOEWCAYLIYKIAAAVYRWYKKEE -PHYKARMAPALCOO 234 -GIMVSINEPHTICHLGYSIGULAFGIKG-DGKOEWCAYLIYKIGAAWWILYDEVGRKWOEYOR KVOITTITA 233 -KYMITFNERKOTGGGOGAALGDAFTNS-SGICGEVICHNUTKAAAWYNWYKKEE -PHYKARMAPALCOO 235 -KYMOTFNERKOTGGOGYGAALGDAFTNS-SGICGEVICHNUTKAAAWWILYDEVGRKWOEKVOG VUTITA 233 -KYMOTFNERKOTGGOGYGAALGDAFTNS-SGICGEVICHNUTKAAAWWILYDEVGRKWOE VOUCCG 234 -KYMOTFNERKOTGGOGYGAALGDAFTNS-SGICGEVICHNUTKAAAXWHYNNVGRYKKEE CVGVUUTSA -KYMOTFNERKOTGGOGYGAALGDAFTNS-SGICGEVICHNUTKAAAXWHYNNVGRYKKEE CVGVUUTSA -KYMOTFNERKOTGGOYGAALGDAFTNS-SGICGEVICYHUVIKAAAXMHIYDKEBRAQOKKKVGUVUCCG 234 -KYMOTFNERKOTGGYGGYGAPALQS-PGEABYLOVNULAAAAWHIYDKEBRAQOKKKVGUVUCCG 234 -HYMUTINEPYTC

Fig. S7. (Continued)

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Myrosinase	¢ VVE-AINSSE-BIARARRYYEECCEVEFHEISKCEWEDIVEKRIAHROILECRSSSRIPOIEPEDERLWKEAOEYIAINHEFTIMAANAEEASYI)-EINYKHDVRVNLFNDPSWKISVLGW-AICE 354
GH1-1 GH1-8	AEP-ATKKPE-DIAAAEROLTECCIFFHBLFVCOMPSLVVERVAERSKKECYKKSRLPKETOEEIDETRCTHDELAFNHYYTFLWADEPEAPYN YEP-ETNKTA-DIOAADROCOFECCWYYHEVFICOMPSVVVERVAERSKKECYKKSRLPKETOEEIKFURCTHDYLAWNHYYTFLAADEPEAPYN	I-RTALENDVRAYNSKSPDWPVSSINW-NIVP 359 I-HTDYDNDVRWINSRSPDWALASNKY-AIVP 356
GH1-3	YBP-ETKYPD-DIEAGNRAQOFTCGWYLWPIFN-GDWEKVVKDRIAERSYKEGYTKSRLPOFTPKBIEFIKGTYDYLGINHYSTFLTSDEPEAPYN YBD-ASNE DE-DWEADE BLYOEFCCWYLEDTCO-COMPENYWEYDERSE DE GYMRSEL DSETASEVWEMECTODE (WMEYSTTCESNYOFSSY	I-DTDYGNDIRIVTTRMPRWGVGANNK-AVVP 353
GH1-21	IEP-KSNSKE-DEEAANROREFKOGNYHEIFIGOMEPIVKERIESRRLKAHIKEPRLPKETPEEIEYIKGSADFIGINHYYTALAEDLKESDPI	I-VMSYGNDVGTVNSFKPSWIVENNGLFSIVP 358
GH1-2 GH1-16	FBP-DSKKLD-DVAARERWOONFGAVGALINL-GDEPKEMKSNIAERSLKOGEFKSKUPAFTOUEIEFLINNTUDFUGVMEYTSAWWRMKIDTEK ABP-DSKDKA-DVEAR <mark>B</mark> RLIQFTYGWYAH <mark>P</mark> VTHGDYBEVMKOF <mark>V</mark> GERSAKQGFSESRLPKE <mark>SKABIHGMKNTVDFLGLNYY</mark> TSVMAKHKNDEEKT	K-EISWAADAEIYAYQKPEWEGAASSWLKVTP 356 F-AIDWDQ <mark>D</mark> SE <mark>V</mark> DTYFKPEWPSTASS <mark>W</mark> LKIVP 356
GH1-13 GH1-9	MƏR-ASDSKA-DIARSERQIQEMFCWFANDILNGDYEWQMKKYIAERSAIQCFSRSRLEEFKEDIYIKGTADFLGVNFYTSNMVKEKNDSKR WERENSTDVE-DQQASETMLQETFCLYANDLHAGDFPDIVKNKVLSRSLAQCFQASRLPAFTDAERRLIKCSYDFGVNYYSSALVTPERDPDPF	4-EV <mark>SWEADAEAIRYHKNEWV-YSTSWLRVTP</mark> 355 2-SMGYKP <mark>D</mark> SE <mark>V</mark> RSWFDPSWEKGASSWLVVTP 359
GH1-20	YBEADSWNIG-DOLASOLKLHETYCLWYNEIVY-GDEBWAVKSIVKKRSLAGGYNRSRLEESSOOKELVKGTYDELGLWYTARYTKEDPDTPVI FED-ETNSTE-DIEASEEDWAGTWEUIGERIWE-COVERIWETWEIVANSKI EGYNGSTOFTSEETEFWEGTUNEEAANSYTTSWEETPEAPI)-GKGYDFDSETTTFVDPHWEKGASFWLYVVP 358
GH1-19	YEP-ETNSTA-DIEAANTKLOESWEFFEHEIVKGOMPEVMKRRIAMRSREEGFEKSRLPEFTEEELEFIRGTSDEFSANTYTTSIIRAEAEPPFC	G-EPTIETDVGVFEYQRPEWQGAASSWLKVTP 342
GH1-14 GH1-15	FLP-ASNSSA-DEEAADKWLHETIGVYANEITVGDEEEVVKSKVAARGAAGGKUUSKUPELTYPELTYINGHEDFEGLWYTGASVAQANEDPPVU HLP-ATDSK <mark>E-NRTAAETQQLEQLEIYANE</mark> IYLGDYEEIVK <mark>ERIAARSKAEGRSQSRLPAFTRDEIDFIRGT</mark> YDFALWVYSAYLVNSIAEPPVU	INPPSKWODOAVTTSMPSDWESTSOOFTKVVP 357 IDPPSNWADIGVNTYQPDDWEKTSAEHIKVVP 357
GH1-18 GH1-25	FCE-ASNTTE-DKKAAEQAFHEELCWYANEVHYGYYEDVMKSRIAARSAAEGRNHSRLEEFTAEEKAQLINSTDEFALNTYTGNLWSALPEPAI YEEEDPGNPD-DVAAVDSKLLEGIGIYLHELVFGYYEDVVKEKVKRRSEVCEFQRSRLEEFNDDEKLKLKKCFDFIGLNYYTSGIAKADRSNPE-	PDPP <mark>S</mark> RFGDMG V NNFYDKSWETTTLDWFKVAP 356 GKGCFVDCEAIEYQAHCWESCALFWLKVVP 358
GH1-6 GH1-7	YEP-STNSKE-DAIASDTKLOEVHGYYGPVVNGNMPDVMIKNVADISRKOGFNESRLPAFTEEBOKLIKGTYDFAINHYTTYMWKARTOEVI- YEP-GSDRDE-DKAAABBATOEDICHYGNELFNGDMPETVKREVAFRSEREGENTSRLPEFTKEOTDYTKGTSDYLAINHYSTLLTNHAPDAPIC	TV <mark>S</mark> WDDDSKVDIYQKSEDIWKPAAIDWFRIVP 365 S-TPSFDKDKSVALSHRSDWTOGSADWFFDVP 358
GH1-23	SEP-GSDSDA-DRAAABRATOFEVGIYANPVFNGDMEEVVKRVAERSKMEGYEESRIPAESEREJAYIKGTSCYLAINTYSTMVWSAAGEAPL- avd-msrkab-draaasorwoervegyappiyacover interataresaasorekyspiperenderson interativeytinvityataeppit	TASFEHDKNVRYWAEPDWPKDGPWWFYDLP 356
GH1-24	NIP-INNTEENDKLAAERRLOFDFGLYANPLVFGDMPEVVKERVKERSSRENFASSRLPAFTEEQKRMINGTLDYLAVNFYDSKLUGDAKEADNS	S-TYSYNNDVRVLIVRNTSLP-AAIDGNTIYA 353
GH1-22 GH1-5	NIP ASJSRE DOCHRENKLODPEGERNELHE - GORESVUNKVOERANNAFIESKUPVETDOCHLENGSDEELVE WOLKNGOARDADIS YEP-ADSGKE-HLEAAEIARESEVGMELNELYHPIGDEEVVKREVETVSKIEGYPESILESETREEIDLMKGSCDFIGANIYTSFIVKRKDDSLFI	D-GT <mark>S</mark> TLR <mark>D</mark> EGAEIFQDPS <mark>W</mark> KGSASS <mark>W</mark> LKVRP 354
GH1-12 GH1-11	YBA-GSFAPE-DIIAAERTREKIGSIMNPIFHTUGNYERIMRERVYNVSREEGYLQSRLPILSPODIDYLOGTYDEAGINIDTTFLURONTEKTN- FOE-KTRFDE-DIEAVATRYOEHAGWFAHEIYKGDYEDVMIERVGKISOLGEFSESRLEKETRKDIRWIKGTSDEFAFGYFMGFYURANSNEIPI	ETATKKDLNVTFYQDKSMTKTNTDMLRVVP 374 PP <mark>S</mark> FGN D AGSKIKLMNSDVA 365
GH1-26 GH1-27	FBP-ASESQS-DIDAAERCOFOICWFAHPIYVGDYPPLMIETIGOISESOCFYDSRLPRETRKETOWIKDHDEFALSYFKTMLVTDNEDQYYI 	SSPSYYS289 SPPVSWEEDAOMOVYOKSTMKTAAIDMFKIVE 116
	▼	
Myrosinase	FGVRELEKHLKEQYGNEPTIFASIGVADDGTSL-KDDIRTEYYQGYFCYTLEAMQIDDWNVAIIPWSLMDNLEWAKGY	IYHFGLIGIDFYNDPTLTRRIKDSTYFYK 461
GH1-8	NGVRRVINALKOVIGNIMU I ITEMGTSDD-GSSI-NDYERINFYCTYFCEILEAMOI DEVRUIGLSVMSIMDNFEWNAGY	ASHFGLYNIN HNDITLERTPKRSVGFFK 463
GH1-10	NGVRAVUVALKEIFGUORHEIFBIGLAUNGISD-DDIDKIDHISUGICEILEAMUVICVUVFGISINSLODNEMISGI TGVRRLUNLEKERVNDEDVMETEIGVADDGTTL-RDDIRIRYFRSYFCNILEAMOVDKVSVVGIIFWSAMDSFEWHRGY	EARFGEYAIDFYNDVILIRRYARSVEFMA 480 EARFGFYAIDFENDPQLLRRPKMSVNYYK 463
GH1-21 GH1-2	DCVRKVUNAIKKNUGDHEULUTELCVTEDGSSL-NDNIRIDFFTDYICNILSAVYEDEVNVTGVIAASLMDSFEWSNGY AGLRKLUIALKNEYGNVPUITENCYSENGTTL-EDDRRINYQTHISNLRDAMD-DCVNVFGYTAASEMDNFEWLAGY	KSHFGFYNINI-TDPNKTRKPKKSAKFFR 464 TERFGLYH <mark>VDF</mark> -STPN <mark>RTRTPKKS</mark> VNYYR 461
GH1-16 GH1-13	NGMEKILNAVNKNYEGLPIIVTENGVSENGTTI-EDDVRIDYYRDHISGVRDALD-DGNNYIGYTANSMMDNFEWTRGY NGIGKLMRALKKTYGDVPHAITENGVANAGDTI-DDZIRINYYRDHISHVRDALD-DGINIIGYTANSLMDNFEWQTGY	SEKFGLYSVDF-NDPERKRTPKKSVSFFK 461 VDRFGLYYVDE-NSPNRTRIPKKSVRFFK 460
GH1-9 GH1-20	MGARKLEKAIAGANGNEKUYUTENGYSDFNGTEDDGTR <mark>UKNIQCYL</mark> SSIKDAMDED <mark>KUNUKGYTUWSLMDNE</mark> GWLFGY	TNKFGLFCVDF-DDPKRTRKPKNSAKFYK 466
GH1-17	NGMEKLUKATIKNEVSNEPTITTENGYADSTGEL-EDTIRVNYIRUYISYIRDAMEYDCVNYIGYSYNSLMDNFEWVSGY	TAKFGVINVNF-TDPERTRTRKLSSYYYE 456
GH1-14	NTVRSLINAIKNTVNNEGIIITENGYPSKGDKDDRRLYNIRGYLSYIRDTMIEDCVSULGYTVN <mark>SEMDNFEWT</mark> SGY	TQKYGLYEVDF-SSPNRTRTIRPSGEFYR 461
GH1-15 GH1-18	MTIEPIENALASEENNEGIVITENEYPSMNGDKUDRELYEIKAYISYIKTEEDEVKULSYIVAHIDDNEEWESGE MGMBVLPKAVKDTYGDPAIVVTENGYPDHGGU-DDAKRLKUHRDYISYIKTDAMIEDDVKUEGYTAASIMDNFEWASGY	TQKFGLYRVDF-KTLKRIRTPRESSIFNG 462 TQKFGLYS <mark>VDF-</mark> ESDAKTRTP <mark>K</mark> SSAKYFR 461
GH1-25 GH1-6	KCIRHVUKAIIDHFERBLUVITENGVADNGEL-EDVARINUIKRHUSQVKDAIDLDNCNVKGVAVNSULDNFEWREGE NGFGKLURAMRKTUGNIEUVITENGVSDKTGIU-KDQTRIDFLRAVMSHMLDAIHEADUNVTAVILATIMDNFEWTIGY	RPRMGLIFVDY-RSPCRRRTPKK 457 IGKS <mark>GLY</mark> YVNR-TDPALPRIPKD <mark>S</mark> AKYYS 471
GH1-7 GH1-23	NGLENILMALKNINNBENVITENGFSDITGTU-EDENRIVULGHUSACLDAIFADKVNLIGYIVNSIMDDWEWIGGY NGLEKFUNALKRINNBENVVTENGVVIGDDTU-EDDKRIHUFRCYLSACLDAIYKDNVNLIAUTANSIMDDWEWIGGY	TSFLGMYRVNF-TDPARPRIKRKSADYFT 464 TSFLGLYKVNF-TDPERPRIKRKSADFFA 462
GH1-4 GH1-24	- GYTDLARAISKEYNNEDIILFENGYGGSSKDU-EDPERISKIQNSLSRFKDALD-ECVKLFGYCVWSLMDNYEWNGGY	TVKYGIHOVDF-SDKNRTRTAKKSAKWYK 453
GH1-22	DELESSELOAVDKTYNKEK ILLIGGNG I PDDGSTL-VDNDRLYYLAGHINALLKS I YDDKI DVFGYTAMSI I DDFEWDGY Dodywyl carwydynnaddynyddyn conference a bride i dyf y gwygnyl chindren y gwygnyl carwyddi i dynerad so	RLHYGLYSVNF-TDSNRNRTAKLSVKYYK 461
GH1-12	Devekvekavkova v standar v stan Devekvekavkova v standar v stand Devekvekavkova v standar v stand	FPDLSREKYGLYHVDF-SDANRKRTARKSAIWYK 499
GH1-11 GH1-26 CH1-27		
0.11 27	nel annel Annur Bail - 515 anne - 561 anne - 561 anne an 1961 ann ann ann an 1961 ann an 1961 ann an 1961	
Myrosinase	NVTTNKRINCDNVK	475
GH1-8	MLNKTRKH	505
GH1-10	ALIANKUDERMINKVWPPLSWGPIGKEFFDIRHKCERKWARKNIVFSN	486
GH1-21 GH1-2	DLSQNRKUTQDKPNRDWPRPF	485 477
GH1-16 GH1-13	QFLARCHVEESKGVAA	478 495
GH1-9 GH1-20	KLIE <mark>RRCI</mark> VEK©E	479 475
GH1-17		470
GH1-14	KVCARCHVD-E01	475
GH1-15 GH1-18	KVSKNKCHVEKOK	475
GH1-25 GH1-6	KIIKOKCUIDDOLNE	457 486
GH1-7 GH1-23	GIMKARCIVDESKOVE	480
GH1-4		467
GH1-22	TILENRALF DEPHIOSISISITISTISTISTISTISTISTISTISTISTISTIST	556
GH1-5 GH1-12	KVVKERKIVP	469 513
GH1-11 GH1-26	HVCTYGCTDYPCPPFYYQQFEINLNIDSSLIINS	505 289
GH1-27	NVIKARCHIDSOVEN	239

Fig. 57. Predicted amino acid sequence alignment of putative GH1 β -glucosidases and the myrosinase from *P. striolata*. Amino acids known to be involved in substrate recognition and hydrolysis from previously characterized β -glucosidases are marked with a lozenge (\diamondsuit) and a black triangle ($\mathbf{\nabla}$), respectively. Glu413 (numbering according to beetle myrosinase sequence) acts as a nucleophile/base, and Glu170 acts as an acid catalyst. The relevant amino acid residues are not conserved in all predicted enzymes shown here. In GH1-5 and GH1-26, the residues corresponding to Glu170 (proton donor) are replaced with Thr and Ser, respectively, and both catalytic Glu residues are replaced with residues with different properties in GH1-11 and GH1-12. Whether these enzymes are active glucohydrolases needs to be determined, but it does not seem likely. The threshold for similarity/identity shading was set to 60%.

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Fig. S8. Majority-rule tree inferred from maximum likelihood analysis of insect GH1 enzymes tested with 1,000 bootstrap replicates. The tree was rooted using a bacterial β -glucosidase sequence from *Clostridium cellulovorans* as an outgroup. Bootstrap values and, in addition, posterior probability values from a Bayesian analysis using the same dataset are shown next to each node (**, not supported in the Bayesian phylogeny). Clades containing enzymes with β -thioglucosidase activity are highlighted with a box. Putative β -glucosidases from *P. striolata* that were heterologously expressed and tested for myrosinase activity are shown in bold. Sequences included in the analysis colleoptera: *Dpo, Dendroctonus ponderosae* (14 sequences); *Pst, P.striolata* (28 sequence); *Tca, T. castaneum* (14 sequences); and *Tmo, Tenebrio molitor* (1 sequence); Hemiptera: *Api, A. pisum* (9 sequences) and *Bbr, Brevicoryne brasicae* (1 sequence); Diptera: *Dme, Drosophila melanogaster* (1 sequence); Lepidoptera: *Sfr, Spodoptera frugiperda* (one sequence); Bacteria: *Cce, Clostridium cellulovorans* (1 sequence).

Table S1. Glucosinolate profile of field-collected P. striolata used in feeding experiments

Compound	P. striolata control I (31 d)*	P. striolata control II (49 o		
Allyl	294.9 ± 50.1	_		
4MTB	284.4 ± 17.3	309.1 ± 22.3		
4MT3But	2,930.6 ± 227.9	2,124.8 ± 170.2		
3MSOP	196.2 ± 29.2	128.5 ± 26.1		
4MSOB	872.7 ± 156.4	644.0 ± 351.3		
4MSO3But	4,620.9 ± 563.9	2,080.1 ± 388.6		
I3M	1,955.5 ± 147.8	2,117.7 ± 226.2		
40HI3M	13.9 ± 2.6	_		
4MOI3M	1,307.9 ± 76.1	1,445.1 ± 129.3		
1MOI3M	307.3 ± 28.4	336.3 ± 25.7		

Mean glucosinolate concentration, pmol \times mg⁻¹ per beetle \pm SEM

I3M, indol-3-ylmethyl; 1MOI3M, 1-methoxyindol-3-ylmethyl; 4MOI3M, 4-methoxyindol-3-ylmethyl; 4MSO3-But, 4-methylsulfinyl-3-butenyl; 3MSOP; 3-methylsulfinylpropyl; 4MTB, 4-methylthiobutyl; 4MT3But, 4-methylthio-3-butenyl; 4OHI3M, 4-hydroxyindol-3-ylmethyl.

*Reference group (day 0) for *P. striolata* transferred to *B. juncea*, *B. rapa*, and *S. alba* fed for 31 d on *A. thaliana* myb28myb29 (n = 12).

[†]Reference group (day 0) for *P. striolata* transferred to *A. thaliana* Col-0 fed for 49 d on *A. thaliana myb28myb29* (n = 12).

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		B. juncea			B. rapa			S. alba		A.	<i>thaliana</i> Col-0	
Compound	Undamaged	Feeding- damaged	Statistics*	Undamaged	Feeding- damaged	Statistics	Undamaged	Feeding- damaged	Statistics	Undamaged	Feeding- damaged	Statistics
Allyl	914.3 ± 160.3	2182.1 ± 487.3	t = 3.2,	I	I		I	I		Ι	I	
3-But	25.2 ± 5.7	114.5 ± 31.6	t = 0.01	426.3 ± 60.1	727.3 ± 147.3	t = 2.3,	I	I		I	I	
4-Pent	I	Ι	<i>P</i> = 0.005	6.2 ± 0.8	52.2 ± 12.5	P = 0.055 t = 5.6,	Ι	I		Ι	I	
20H3But	I	I		74.8 ± 9.4	44.5 ± 4.1	P < 0.001 t = 2.2,	I	I		I	ļ	
4MTB	I	I		4.7 ± 0.6	2.8 ± 0.2	P = 0.067 t = 2.0,	I	Ι		52.2 ± 3.1	21.9 ± 7.1	t = 3.9,
3MSOP	Ι	Ι		Ι	Ι	P = 0.080	Ι	Ι		96.7 ± 9.3	178.5 ± 7.4	r = 0.004 t = 6.4,
4MSOB	I	I		87.1 ± 8.2	89.9 ± 16.7	t = 0.2,	I	I		696.4 ± 65.0	$1,409.9 \pm 80.1$	P < 0.001 t = 7.0,
5MSOP	I	I		14.1 ± 0.9	40.9 ± 11.5	P = 0.878 t = 3.5,	I	I		22.0 ± 2.6	53.5 ± 3.1	P < 0.001 z = -2.7,
7MSOH	I	I		I	I	P = 0.010	I	I		9.7 ± 0.9	33.1 ± 3.2	P = 0.007 t = 6.4,
8MSOO	Ι	Ι		Ι	Ι		Ι	Ι		48.0 ± 2.6	96.6 ± 13.8	P < 0.001 Z = -2.5,
Benzyl	n.d.	21.0 ± 2.7		n.d.	42.1 ± 0.5		22.2 ± 9.6	162.8 ± 28.4	<i>t</i> = 6.1,	I	I	P = 0.013
2-Phenylethyl	I	I		12.3 ± 2.1	5.7 ± 1.8	t = 2.0,	2.3 ± 1.4	5.5 ± 2.1	P < 0.001 t = 1.3,	I	l	
40H-Benzyl	I	I		I	I	P = 0.086	$1,312.9 \pm 258.0$	3,752.8 ± 238.7	P = 0.239 t = 6.0,	I	l	
40HI3M	16.2 ± 2.2	22.9 ± 6.0	z = -0.9,	4.9 ± 0.6	0.8 ± 0.5	t = 4.3,	1.4 ± 0.6	1.0 ± 0.5	P < 0.001 t = 0.0,	4.7 ± 0.8	9.8 ± 1.3	t = 3.4,
I3M	14.1 ± 2.4	68.1 ± 21.3	r = 0.300 t = 3.8, r = 0.007	21.1 ± 2.5	101.7 ± 45.8	r = 0.004 t = 2.7, p = 0.022	2.6 ± 0.6	3.2 ± 0.3	r = 1.000 t = 0.6, p = 0.623	113.8 ± 9.7	324.7 ± 29.0	r = 0.008 t = 7.2,
4MOI3M	4.7 ± 0.6	12.9 ± 1.6	t = 5.8, t = 5.8, $P \neq 0.001$	8.1 ± 0.6	13.8 ± 3.7	t = 2.2, t = 2.2, P = 0.061	6.3 ± 2.3	3.1 ± 0.8	z = -0.7947, z = -0.7947, z = -0.477	34.6 ± 1.6	40.4 ± 4.4	t = 1.2, p = 0.254
1MOI3M	2.0 ± 0.2	8.0 ± 1.8	t = 4.9, P = 0.002	9.0 ± 1.9	677.2 ± 97.5	z = -2.2, P = 0.028	n.d.	2.4 ± 0.3		8.0 ± 1.0	134.6 ± 9.6	t = 15.6, P < 0.001

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Table S3. Summary of identified *P. striolata* GH1 β-glucosidases

	GenBank	Signal		Molecular	Isoelectric	Predicted	RPKM,	log ₂ *
Name	accession no.	peptide, aa	Length, aa	mass, kDa	point	N-glycosylation sites	Female	Male
GH1-1	KF377828	19	510	58.85	8.75	4	4.8	4.9
GH1-2	KF377829	17	477	55.06	5.56	3	6.2	6.1
GH1-3	KF377830	22	509	59.61	6.46	4	8.8	0.5
GH1-4	KF377831	19	467	53.12	5.45	3	5.1	5.1
GH1-5	KF377832	—	469	53.72	6.00	2	4.0	2.6
Myrosinase	KF377833	20	475	54.92	5.82	3	1.5	2.0
GH1-6	KF377834	18	486	55.85	5.41	3	5.7	5.6
GH1-7	KF377835	18	480	54.81	5.41	3	7.2	6.6
GH1-8	KF377836	20	505	59	8.19	6	6.3	6.6
GH1-9	KF377837	22	479	55.09	5.31	3	7.0	6.8
GH1-10	KF377838	18	486	56.69	6.54	2	4.9	-3.5
GH1-11	KF377839	_	505	58.07	5.25	3	5.8	-0.7
GH1-12	KF377840	_	513	59.73	6.41	4	2.3	2.6
GH1-13	KF377841	19	495	57.54	5.96	2	6.1	6.2
GH1-14	KF377842	17	475	53.99	4.95	3	6.3	6.0
GH1-15	KF377843	15	475	54.53	6.16	3	4.2	4.0
GH1-16	KF377844	18	478	54.92	5.29	0	6.4	6.5
GH1-17	KF377845	18	470	54.48	5.01	5	5.1	4.7
GH1-18	KF377846	21	475	54.55	6.10	3	4.4	2.4
GH1-19	KF377847	17	460	53.28	5.00	5	6.5	6.2
GH1-20	KF377848	18	475	55.15	8.72	4	3.2	3.1
GH1-21	KF377849	21	485	56.13	6.89	3	-5.3	5.3
GH1-22	KF377850	17	556	62.28	4.99	6	6.7	6.9
GH1-23	KF377851	16	478	54.62	5.20	3	6.0	5.4
GH1-24	KF377852	18	520	59.12	5.39	5	5.5	5.2
GH1-25 [†]	KF377853						1.8	2.6
GH1-26 [†]	KF377854						6.9	-4.1
$GH1-27^{\dagger}$	KF377855						7.6	7.2

*Reads per kilobase of transcript per million mapped reads (RPKM; log₂) values are provided to compare relative expression in female and male *P. striolata*. [†]Partial sequence.

Other Supporting Information Files

Dataset S1 (XLSX) Dataset S2 (XLXS) Dataset S3 (XLXS)

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