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Impact of glucosinolate structure on the performance of the crucifer pest *Phaedon cochleariae* (F.)

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Summary

Glucosinolates (GS) are sulfur-rich secondary metabolites found in the Brassicaceae and other related families of the order Brassicales. GS consist of structurally-related compounds with different side chains. To explore the possibility that various side chain confer divergent biological activities to individual GS, we have investigated the performance of the specialist pest beetle, *Phaedon cochleariae* (F.) on *Arabidopsis thaliana* L. mutants and Columbia wild-type (WT) which differ in the main group of GS. Plant lines of *A. thaliana* altered for the expression of *MAM3*, because of the introduction of an over-expression construct of *MAM3* (*mam3*$^{+}$) or containing double knock-outs of *CYP79B2* and *CYP79B3* (*cyp79B2*$^{-}$/*cyp79B3*$^{-}$) were used for the study in comparison to the WT.

A. thaliana genotypes differed in their GS profiles. The highest GS content was present in the WT followed by *mam3*$^{+}$ and *cyp79B2*$^{-}$/*cyp79B3*$^{-}$. A modified aliphatic GS content was detected for the *mam3*$^{+}$ as compared to the WT lines. Furthermore, indolyl GS were completely absent in *cyp79B2*$^{-}$/*cyp79B3*$^{-}$. The percentage weight increase of larvae raised on each of the three plant genotypes was significant different. Larval performance was poorest on plants of *cyp79B2*$^{-}$/*cyp79B3*$^{-}$ and best on WT, but there was no significant difference found in percentage weight increase on *mam3*$^{+}$ and WT. There was no correlation between the weight increase of the larvae on genotypes and induced levels of aliphatic, indolyl, and total GS. However, the poor performance of beetle larvae on *cyp79B2*$^{-}$/*cyp79B3*$^{-}$compared to WT and *mam3*$^{+}$ might be explained by comparable high aliphatic GS levels of this mutant, a different induction of secondary metabolites, and the absence of indolyl GS. Basic knowledge about the relationship of GS structures and their insect pests may help in further resistance breeding of crucifer crops.

Introduction

Glucosinolates (GS) are sulphur-rich β-thioglucosides present within the Brassicaceae and other related families of the order Brassicales (RODMAN et al., 1996). All GS contain a common core structure, but have variable side chains including aliphatic, aromatic, or indolic structures (WITTSTOCK and HALKIER, 2002; KLIEBENSTEIN et al., 2005). To date, more than 120 GS have been identified in plants (FAHEY et al., 2001), including 34 compounds present in the model plant *Arabidopsis thaliana* L. (KLIEBENSTEIN et al., 2001). In plants GS and their hydrolyzing enzymes, β-thioglucosidases, which are also called myrosinases, are stored in different cell compartments or specialized cells (LÜTHY and MATILE, 1984; KOROLEVA et al., 2000). But when the plant is damaged, hydrolysis occurs and biological active products such as isothiocyanates, thiocyanates, and nitriles are released.

GS have dual roles in plant-insect interactions. Although they function as defense compounds, several insects that are specialists have life cycles that are closely linked to the presence of such secondary compounds in their brassicaceous host plants (RENWICK, 2002). They are known to stimulate feeding in a number of insects that feed exclusively on crucifers (DAVID and GARDINER, 1966; NAULT and STYER, 1972; TANTON, 1977; LARSEN et al., 1985). Brassicaceae species and individual plants vary within concentration and composition of GS (FAHEY et al., 2001; KLIEBENSTEIN et al., 2001). Additionally, many brassicaceous plants produce higher levels of indolyl GS after herbivory (KORTISAS et al., 1991; BODNARYK, 1992; GRIFFITHS et al., 1994; BAUER et al., 1998; AGRAWAL et al., 1999a). GS and/or their breakdown products have a variety of effects upon organisms that come in contact with them, but do not always control the interaction between plants and their potential herbivore or invading microorganisms (CHEW, 1988b). Distinct GS can have different effect on the same insect herbivore. For example, *p*-hydroxybenzyl GS is involved in plant resistance (antixenosis) against feeding by the flea beetle, *Phyllotreta cruciferae* (Goeze), in seedlings of yellow mustard, *Sinapis alba* L. (BODNARYK, 1991).Whereas, allyl GS and indol-3-ylmethyl, which are present in seedlings of Indian mustard, *Brassica juncea* (L.) and oilseed rape, *Brassica napus* L., respectively, are not determinants of feeding behavior by *P. cruciferae* (BODNARYK and PALANISWAMY, 1990).

The mustard leaf beetle, *Phaedon cochleariae* (F.) (Coleopterans: Chrysomelidae), is an insect pest of crucifer crops that is common to Europe (FINCH and JONES, 1986). When this insect emerges in large numbers, it can quickly devastate crucifer fields with especially severe effects on young seedlings. Hence, a study of the insect/host-plant interaction including the feeding behavior can lead to agriculturally relevant management strategies. TANTON (1977) and NIELSEN (1978) found that *P. cochleariae* responded to a variety of GS in feeding bioassays, with distinct preferences for the aliphatic allyl GS (sinigrin) compared to the aromatic compounds: p-hydroxybenzyl GS (sinalbin) and benzyl GS (glucotropaeolin). However, these studies have limited insights into the impact of GS structure on the larvae and beetle performance of *P. cochleariae*. We have taken this opportunity to investigate the effect of different GS classes on the feeding and growth of the *P. cochleariae* larvae by using *A. thaliana* genotypes with desired GS profiles.

Materials and methods

Plant cultivation and experimental conditions

Seeds from single seed propagation were sown on petri plates containing solidified Murashige-Skoog media. After stratification for three days at 4°C, germinated seedlings were transferred to 7 x 6 cm pots filled with sterile potting mix (Gramoflor). Plants were kept in a growth chamber at 21 ± 1°C, 60 ± 5% relative humidity, at 200 µmol m^{-2} s^{-1} light intensity, and on a 10.5 : 13.5 (L : D) photoperiod. Twenty-six days old plants were used for the force feeding experiments.

Insect rearing

The start population of *P. cochleariae* was obtained from Bayer Crop Science (Mohnheim, Germany). This beetle species was initially

reared on pak-choi plants (*Brassica chinensis* L.) following by savoy cabbage (*Brassica sabauda* L.) in the laboratory of the Urban Horticulture section at Humboldt University Berlin. Pak-choi plants were provided as food in insect cages. For egg laying the beetles were provided the severed stem of savoy cabbage. After the beetles laid eggs on savoy cabbage, which was transferred to a new cage for larval hatching. Larvae were fed with savoy cabbage leaves, which were replaced on alternate days until larval pupation. Pupae were transferred to another cage for beetle hatching. This procedure was repeated to obtain a sufficient number of insects all at the 1st instar stage for the bioassays.

Force feeding experiments

Lines (three each) of two different *A. thaliana* mutants with modified GS profiles and the corresponding Columbia wild-type (WT) were used for the bioassays. The first lines were the double knock-out mutant of *CYP79B2* and *CYP79B3* (*cyp79B2-/cyp79B3-*, a gift from J. Chory, The Salk Institute for Biological Studies, California). Further information on the construction of the mutant can be reviewed in Zhao et al. (2002). The second plant mutant lines are characterized by over expression of *MAM3* (*mam3+*) and were generated as described in Textor et al. (2007). First instars of *P. cochleariae* were weighted before release on the plants, one larva per plant. The pots were covered with small cages made from transparent plastic cylinders and fine mesh gauze. After four days pre-conditioning to their respective plant lines, the larvae were weighted and transferred to a new plant of the same genotype. After three days the final larval weight was determined. Ten replicates were made for each of the nine lines of mutants and WT. Four plants of each genotype were harvested following larval feeding at 30 days after planting (first harvest) as well as 33 days after planting (second harvest), frozen in liquid nitrogen and stored at -80°C prior to GS analysis. Data from chemical analysis and bioassays data were analyzed by performing variance analysis with following mean comparison test by using SYSTAT 11.0.

Glucosinolate analysis

Plant samples were freeze-dried and whole plants were grounded. Samples of 20 mg were extracted in 70% methanol following the procedure described by Mewis et al. (2005). Extraction was done in five replications for each treatment and genotype. To quantify GS content, an internal standard *p*-hydroxybenzyl GS (purified from *Sinapis alba* seeds as potassium salt) was added initially to the first methanol extract. The GS of extracts were analyzed as desulfo GS and for this purpose the extracts were desulfated on DEAE Sephadex A-25 mini columns with aryl sulfatase solution (Sigma-Aldrich Corp H 1 from *Helix pomatia*); column wash steps are as described in Mewis et al. (2005). A 40 µl aliquot of each GS extracts was run on a Dionex Summit P680A HPLC system equipped with an ASI-100 auto sampler and a PDA-100 photodiode array detector. GS were separated on a narrow bore column (Acclaim ™ 120, 250-2.1, RP18, Dionex) at a flow rate of 0.4 ml / min and a column temperature of 25°C. A 43 min gradient program with eluents: A) ultra pure H_2O (Milli Q quality) and B) 40% acetonitrile (HPLC grade) consisted of 0 - 1 min 99.5% A, 1 - 8 min 80%, 10 - 19 min 50%, 22 - 28 min 1.0% A, 33 - 36 min 99.5% A. The eluent was monitored for absorbance at 229 nm and GS were identified using purified standards, retention time, and UVVis spectra according to Brown et al. (2003). The GS amount was calculated from HPLC peak areas using response factors of desulfo GS at 229 nm.

Results

Insect performance on genotypes with different glucosinolate profiles

Percentage weight increase of larvae force-fed on each of the genotypes was significantly different in the first experiment (Fig. 1A). Larval performance was poorest on the *cyp79B2-/cyp79B3-* mutants followed by *mam3+*, and WT. There was no significant difference in percentage weight increase determined after feeding on *mam3+* and WT. Although the same trend was observed in the second experiment (Fig. 1B), the differences observed were not significant. To explain the impact of different types of GS on *P. cochleariae* larval performance, simple correlation of the larval weight increase to induced total GS contents were performed. We found that there was no correlation with R = 0.136 between the weight increase of the larvae and increasing levels of induced total GS among all genotypes. The performance of larvae was comparable poor on *cyp79B2-/cyp79B3-* mutants with lowest GS levels. Although a different effect of GS classes was expected, no correlation of larvae performance to induced indolyl (R = 0.231) or aliphatic GS (R = 0.032) contents of genotypes was found (Fig. 2 A and B).

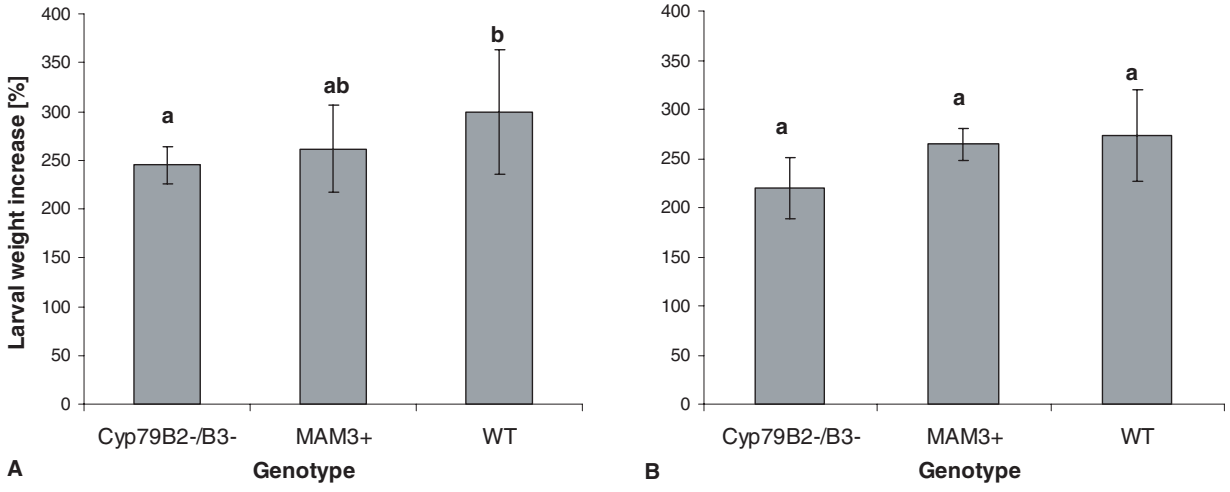

Fig. 1: Percentage weight increase of *P. cochleariae* larvae on different *A. thaliana* genotypes in the first (A) and second experiment (B) (Different letters indicate significant differences among treatments, Fisher's LSD $p \leq 0.05$)

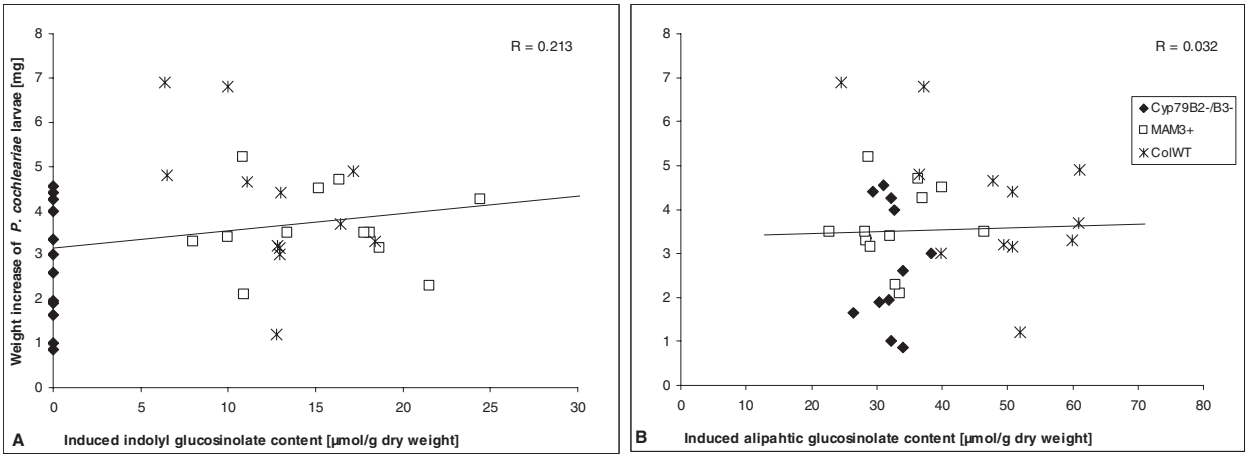

Fig. 2: Correlation between weight increase of *P. cochleariae* larvae and induced aliphatic (A) and indolyl glucosinolate (B) content of *A. thaliana* genotypes (1st experiment)

Glucosinolate induction of *Arabidopsis thaliana* genotypes by *Phaedon cochleariae*

The experimental *A. thaliana* mutant lines differed in their GS profile compared to the WT (Tab. 1). In the WT the major aliphatic and indolyl GS were 4-methylsulfinylbutyl GS and indol-3-ylmethyl GS, respectively. In contrast to WT, 3-methylsulfinylpropyl GS was the dominant aliphatic GS in *mam3*$^+$ and levels of the long chain aliphatic GS, C7 and C8, were increased. Overall, levels of aliphatic GS were lower than in WT but with about similar levels of indolyl GS. The major GS compound detected in *cyp79B2*$^-$*/B3*$^-$ was 4-methylsulfinylbutyl GS followed by 3-methylsulfinylpropyl GS, as in WT, but indolyl GS were complete absent in this mutant. Similar results for constitutive and induced levels were observed for the two experiments.

The accumulation of GS following herbivory damage differed in the three genotypes (Tab. 1). After feeding of *P. cochleariae*, levels of total GS increased in all genotype lines, but the accumulation was only significant with the *cyp79B2-/B3-* mutant (Tukey's HSD test $p = 0.004$). In the WT, a marked increase of indol-3-ylmethyl and 1-methoxyindol-3-ylmethyl GS levels was observed after larval feeding (Tab. 1). Also the major methylsulfinyl GS increased after herbivory of *P. cochleariae*. In the *mam3*$^+$ mutant levels of nearly all aliphatic (except 4-methylthiobutyl GS and 8-methylsulfinyloctyl GS) and indolyl GS increased after feeding by larvae. The major aliphatic GS, 4-methylsulfinylbutyl GS increased about two/three-fold. As with the WT response, a strong increase of indol-3-ylmethyl and 1-methoxyindol-3-ylmethyl GS was observed after beetle herbivory. Only methylsulfinyl GS were induced by larvae feeding in *cyp79B2-/B3-*, wherein this GS increased about two-fold in this mutant.

Discussion

It has been shown that GS and their corresponding hydrolysis products determine plant resistance in crucifers such as *A. thaliana* and *Brassica* sp., especially against generalist insects (Lambrix et al., 2001, reviewed in Kliebenstein et al., 2004). However, GS are known to influence the behavior of insects that specialize on crucifer plants (Renwick, 2002; Mewis et al., 2002). The effects of GS are assumed to vary depending on the attacking insect herbivore as well as the concentration and composition of GS present in the plants. Many studies have demonstrated that GS and their hydrolysis products stimulate feeding in crucifer specialists (Hicks, 1974; Chew, 1988a; Barlet and Williams, 1991; Li et al., 2000). That GS and their corresponding hydrolysis products can adversely affect the performance of specialists as well is reported by Agrawal and Kurashige (2003). They reported a negative effect of higher GS concentrations on the lepidopteron *Pieris rapae* L.

To study the effect of GS classes on the crucifer specialist *P. cochleariae*, we used different mutant lines of *A. thaliana* with modified GS profiles and their corresponding WT. Larval performance in the experiments, measured as percentage weight increase, was best on Columbia WT, followed by *mam3*$^+$, and the double knock out mutant *cyp79B2*$^-$*/cyp79B3*$^-$. This was opposite to detected constitutive and induced GS contents in genotypes, whereby levels were highest in the WT and lowest in *cyp79B2*$^-$*/cyp79B3*$^-$. Furthermore, we did not find a negative correlation of larval performance to increasing total GS contents of genotypes. This differs to other studies, where weight increase of crucifer specialist larvae was slower on plants with higher GS contents (Nayar and Thorsteinson, 1963; Ahman, 1986; Louda and Collinge, 1992; Wolfson, 1982; Agrawal and Kurashige, 2003; Rohr et al., 2006). For example, Li et al. (2000) reported that there was a negative correlation of larvae weight increase in *Plutella xyllostella* (L.) and *Spodoptera eridania* (Stoll) to an increasing allyl GS concentration. Also Rohr et al. (2006) found that larval performance of *Pieris brassicae* L. was negatively related to increasing GS contents in *A. thaliana* ecotypes. In contrast, Kliebenstein et al. (2005) demonstrated that plant feeding damage was positively correlated to increasing levels of aliphatic GS in the crucifer specialist *P. xylostella*, whereas negative relationships of larval performance and increasing GS levels were detected for the generalist caterpillars *Trichoplusia ni* (Hübner) and *Spodoptera exigua* (Hübner). In our present study the insect-plant interaction seems to be complex, but it can be noted that the *P. cochleariae* performance was worse on the mutant which contain only aliphatic GS. Furthermore, beetles feeding induced aliphatic GS to a greater extent in *cyp79B2*$^-$*/cyp79B3*$^-$ and *mam3*$^+$ compared to the WT at the end of the experiment (second harvest, Tab. 1). The study of Siemens and Mitchellolds (1996) with *Phyllotreat cruciferae* (Goeze) and *P. xylostella* showed that herbivory of these insects varied curvilinearly with GS levels in *Brassica campestris* (L.), with maximum herbivory at intermediate GS levels. Such relationship could be also true for *P. cochleariae*.

A. thaliana ecotypes differ especially in their aliphatic but not their indolyl GS structures (Kliebenstein et al., 2001; Reichelt et al., 2002). From the high variability of aliphatic GS it is assumed that structurally different GS and their hydrolysis products have different biological effects. For example, higher oviposition preference by

Tab. 1: Glucosinolate content of *Arabidopsis thaliana* genotypes in the first experiments, control plants and plants after *P. cochleariae* feeding (induction of single compounds is in grey)

Genotype	Glucosinolate	Glucoinolate content (µmol/g dry weight)			
		First harvest		Second harvest	
		Control	*Phaedon*	Control	*Phaedon*
WT	3-Methylsulfinylpropyl	4.34	5.04	4.77	5.76
	4-Methylsulfinylbutyl	26.07	27.47	24.72	36.96
	5-Methylsulfinylpentyl	0.71	0.86	0.72	1.08
	6-Methylsulfinylhexyl	0.13	0.21	0.17	0.26
	4-Hydroxyindol-3-ylmethyl	0.10	0.19	0.27	0.32
	7-Methylsulfinylheptyl	0.97	0.96	0.93	1.12
	4-Methylthiobutyl	1.30	1.29	1.18	1.10
	Indol-3-ylmethyl	0.86	6.51	5.24	10.14
	8-Methylsulfinyloctyl	0.00	0.00	0.00	0.64
	4-Methoxyindol-3-ylmethyl	1.28	1.24	0.32	0.90
	1-Methoxyindol-3-ylmethyl	0.57	1.13	0.00	1.20
	7-Methylthioheptyl	0.10	0.14	0.05	0.09
	8-Methylthiooctyl	0.19	0.22	0.13	0.20
	Total	**36.63a**	**45.27a**	**38.48a**	**59.76a**
mam3+	3-Methylsulfinylpropyl	11.53	12.78	11.46	15.31
	4-Methylsulfinylbutyl	4.22	7.07	3.61	10.13
	5-Methylsulfinylpentyl	0.24	0.42	0.25	0.49
	6-Methylsulfinylhexyl	0.38	0.66	0.58	1.14
	4-Hydroxyindol-3-ylmethyl	0.07	0.14	0.12	0.33
	7-Methylsulfinylheptyl	1.77	2.90	2.14	3.23
	4-Methylthiobutyl	0.35	0.39	0.16	0.34
	Indol-3-ylmethyl	5.59	7.59	6.60	12.79
	8-Methylsulfinyloctyl	1.22	0.29	0.00	0.04
	4-Methoxyindol-3-ylmethyl	1.34	1.42	1.10	1.83
	1-Methoxyindol-3-ylmethyl	0.47	1.94	0.08	0.49
	7-Methylthioheptyl	0.47	0.93	0.63	0.73
	8-Methylthiooctyl	0.75	1.29	0.81	1.54
	Total	**28.38a**	**37.81a**	**27.53a**	**48.38b**
cyp79B2-/B3-	3-Methylsulfinylpropyl	1.68	2.34	2.19	3.68
	4-Methylsulfinylbutyl	9.36	14.91	10.79	21.73
	5-Methylsulfinylpentyl	0.28	0.49	0.32	0.75
	6-Methylsulfinylhexyl	0.07	0.13	0.06	0.19
	4-Hydroxyindol-3-ylmethyl	0.00	0.00	0.00	0.00
	7-Methylsulfinylheptyl	0.32	0.67	0.32	0.86
	4-Methylthiobutyl	1.10	1.03	0.96	0.85
	Indol-3-ylmethyl	0.00	0.00	0.00	0.00
	8-Methylsulfinyloctyl	1.22	2.73	1.68	3.65
	4-Methoxyindol-3-ylmethyl	0.00	0.00	0.00	0.00
	1-Methoxyindol-3-ylmethyl	0.00	0.00	0.00	0.00
	7-Methylthioheptyl	0.06	0.10	0.06	0.06
	8-Methylthiooctyl	0.14	0.22	0.17	0.15
	Total	**14.21a***	**22.60b**	**16.53a**	**31.93b**

*different letters indicate significant differences among treatments, Tukey's HSD test $p \leq 0.05$

Hellula undalis (F.) was noted for aliphatic alkenyl GS than indolyl GS (MEWIS et al., 2002). Also KLIEBENSTEIN et al. (2002b) found evidence for diverse biological activities of GS by studying their variation and impact on plant resistance against the generalist *T. ni*. The authors suggested that GS with alkenyl side chains were more of a deterrent to the test insect than GS with non-alkenyl side chains. ROHR et al. (2006) observed a correlation of side chain structure of GS in *A. thaliana* ecotypes and the performances of a generalist and specialist lepidopteron, *S. exigua* and *P. brassicae*, respectively. Here larval weight gain was greater on the 3-hydroxypropyl GS-containing ecotypes than on methylsulphinyl GS-producing ecotypes. Furthermore, GIAMOUSTARIS and MITHEN (1995) reported an increasing damage of *Brassica napus* L. plants by the cabbage stem flea beetle, *Psylliodes chrysocephala* (L.), with plants containing GS with shorter side chains and increased levels of hydroxylation. Our initial results with *P. cochleariae* do not support the notion of different responses to structurally distinct classes of GS by a specialist. However, in this study *P. cochleariae* fed on only a limited number of GS, which probably represents a subset of all the GS that the species may encounter. However, further studies with different specialist insects are needed to identify general effects of GS structures on feeding behavior that could be employed by plant breeders to develop insect resistance crop plants.

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References

AGRAWAL, A.A., 1999a: Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. Ecology 80, 1713-1723.

AGRAWAL, A.A., KURASHIGE, N.S., 2003: A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. J. Chem. Ecol. 29, 1403-1415.

AHMAN, I., 1986: Toxicities of host secondary compounds to eggs of the *Brassica* specialist *Dasineura brassicae.* J. Chem. Ecol. 12, 1481-1488.

BARLET, E., WILLIAMS, I.E., 1991: Factors restricting the feeding of the cabbage stem flea beetle (*Psylliodes chrysocephala*). Entomol. Exp. Appl. 60, 233-238.

BAUER, R., STAEDLER, E., MONDE, K., TAKASUGI, M., 1998: Phytoalexins from *Brassica* (Cruciferae) as oviposition stimulants the cabbage root fly, *Delia radicum*. Chemoecology 8, 163-168.

BODNARYK, R.P., 1991: Developmental profile of sinalbin (*p*-hydroxybenzyl glucosinolate) in mustard seedlings, *Sinapis alba* L., and its relationship to insect resistance. J. Chem. Ecol. 17, 1543- 1556.

BODNARYK, R.P., 1992: Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard. Phytochemistry 31, 2671-2677.

BODNARYK, R.B., PALANISWAMY, P., 1990: Glucosinolate levels in cotyledons of mustard *Brassica juncea* (L.) and Rape, *B. napus* (L.) do not determine feeding rates of flea beetle, *Phyllotreta cruciferae* (Goeze). J. Chem. Ecol. 6, 2735-2746.

BROWN, P.D., TOKUHISA, J.G., REICHELT, M., GERSHENZON, J., 2003: Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana.* Phytochemistry 62, 471-481.

CHEW, F.S., 1988a: Biological effects of glucosinolates. In: Cutler, H.G. (ed.), *Biologically Active Natural Products: Potential Use in Agriculture,* American Chemical Society, Washington, DC, 155-181.

CHEW, F.S., 1988b: Searching for defensive chemistry in the Cruciferae, or, do glucosinolates always control interactions of Cruciferae with their potential herbivores and symbionts? No! *Chemical Mediation of Coevolution* (ed. by K.C. Spencer), 81-112.

DAVID, W.A.L., GARDINER, B.O.C., 1966: Mustard oil glucosides as feeding stimulants for Pieris brassicae larvae in a semi-synthetic diet. Entomol. Exp. Appl. 9, 247-255.

FAHEY, J.W., ZALCMANN, A.T., TALALAY, P., 2001: The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56, 5-51.

FINCH, S., JONES, T.H., 1986: Interspecific competition during host plant selection by insect pests of crucifer crops. Series Entomologia (The Hague) Vol. 41, Insect-Plants, 85-90.

GRIFFITH, D.W., BIRCH, A.N.E., MACFARLANE-SMITH, W.H., 1994: Induced changes in the indole glucosinolate content of oilseed and forage rape (*Brassica napus*) plants in response to either turnip root fly (*Delia floralis*) larval feeding or artificial damage. J. Sci. Food. Agric. 65, 171-178.

HICKS, K.L., 1974: Mustard oil glucosides: Feeding stimulants for adult cabbage flea beetle, *Phyllotreta cruciferous* (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Amer. 67, 261-264.

KLIEBENSTEIN, D.J., KROYMANN, J., BROWN, P.D., FIGUTH, A., PEDERSEN, D., GERSHENZON, J., MITCHELL-OLDS, T., 2001: Genetic control of natural variation in *Arabidopsis thaliana* glucosinolate accumulation. Plant Physiol. 126, 811-825.

KLIEBENSTEIN, D.J., 2004: Secondary metabolites and plant/environment interactions: a view through *Arabidopsis thaliana* tinged glasses. Plant Cell Environ. 27, 675-684.

KLIEBENSTEIN, D.J., KROYMANN, J., MITCHELL-OLDS, T., 2005: The glucosinolate myrosinase system in an ecological and evolutionary context. Curr. Opin. Plant Biol. 8, 264-271.

KLIEBENSTEIN, D.J., PEDERSEN, D., MITCHELL-OLDS, T., 2002b: Comparative analysis of insect resistance QTL and QTL controlling the myrosinase / glucosinolate system in *Arabidopsis thaliana*. Genetics 161, 325-332.

KOROLEVA, O.A., DAVIES, A., DEEKEN, R., THORPE, M.R., TOMOS, A.D., HEDRICH, R., 2000: Different myrosinase and ideoblast distribution in *Arabidopsis* and *Brassica napus.* Plant Physiol. 127, 1750-1763.

KORTISAS, V.M., LEWIS, J.A., FENWICK, G.R., 1991: Glucosinolate responses of oilseed rape, mustard and kale to mechanical wounding and infestation by cabbage stem flea beetle (*Psylliodes chrysocephala*). Ann. Appl. Biol. 118, 209-222.

LAMBRIX, V., REICHELT, M., MITCHELL-OLDS, T., KLIEBENSTEIN, D.J., GERSHENZON, J., 2001: The *Arabidopsis* epithiospecifer protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. Plant Cell 13, 2793-2807.

LARSEN, L.M., NIELSEN, J.K., PLONGER, A., SØRENSEN, H., 1985: Responses of some beetle species to varieties of oilseed rape and to pure glucosinolates. In: Sorensen, H. (ed.), Advances in the production and utilization of cruciferous crops (Copenhagen 1984). Martinus Nijhoff, Dordrecht, Netherlands, 230-244.

LOUDA. S.M., COLLINGE, S.K., 1992: Plant resistance to insect herbivores: A field test of the environmental stress hypothesis. Ecology 73, 153-169.

LÜTHY, B., MATILE, P., 1984: The mustard oil bomb-rectified analysis of the subcellular organization of the myrosinase system. Biochem. Physiol. Pflanzen 179, 5-12.

MEWIS, I., APPEL, H.M., HOM, A., RAINA, R., SCHULTZ, J.C., 2005: Major signaling pathways modulate *Arabidopsis thaliana* (L.) glucosinolate accumulation and response to both phloem feeding and chewing insects. Plant Physiol. 138, 1149-1162.

MEWIS, I., ULRICHS, C., SCHNITZLER, W.H., 2002: The role of glucosinolates and their hydrolysis products in oviposition and host-plant finding by cabbage webworm, *Hellula undalis*. Entomol. Exp. Appl. 105, 129-139.

NAULT, L.R., STYER, W.E., 1972: Effects of sinigrin on host selection by aphids. Entomol. Exp. Appl. 15, 423-437.

NAYAR, J.K., THORSTEINSON, A.J., 1963: Further investigations into the chemical basis of insect-host plant relationships in an oligophagous insect,

Plutella maculipennis. Can. J. Zool. 41, 923-929.

NIELSEN, J.K., 1978: Host plant discrimination within Cruciferae: feeding responses of four leaf beetles (Coleoptera: Chrysomelidae) to glucosinolates, cucurbitacins and cardenolides. Entomol. Exp. Appl. 24, 41-54.

QUN, L., EIGENBRODE, S.D., STRINGAM, G.R., THIAGARAJAH, M.R., 2000: Feeding and growth of *Plutella xyllostella* and *Spodoptera eridania* on *Brassica juncea* with varying glucosinolate concentrations and myrosinase activities. J. Chem. Ecol. 26, 2401-2419.

REICHELT, M., BROWN, P.D., SCHNEIDER, B., OLSHAM, N.J., STAUBER, E., TOKUHIA, J.G., KLIEBENSTEIN, D.J., MITCHEL-OLDS, T., GERSHENZON, J., 2002: Benzoic acid glucosinolate esters and other glucosinolates from *Arabidopsis thaliana*. Phytochemistry 59, 663-671.

RENWICK, J.A.A., 2002: The chemical world of crucivores: lures, treats and traps. Entomol. Exp. Appl. 104, 35-42.

RODMAN, J.E., KAROL, K.G., PRICE, R.A., SYTEMA, K.J., 1996: Molecules, morphology and dahlgren's expanded order Capparales. Systematic Botany 21, 289-307.

ROHR, F., ULRICHS, C., MUCHA-PELZER, T., MEWIS, I., 2006: Variability of aliphatic glucosinolates in *Arabidopsis* and their influence on insect resistance. Comm. Appl. Biol. Sci. Ghent University, 71, 507-515.

SIEMENS, D.H., MITCHELL-OLDS, T., 1996: Glucosinolates and herbivory by specialists (Coleoptera: Chrysomelidae, Lepidoptera: Plutellidae): Consequences of concentration and induced resistance. Environ. Entomol. 25, 1344-1353.

TANTON, M.T., 1977: Response to food plant stimuli by larvae of the mustard beetle *Phaedon cochleariae*. Entomol. Exp. Appl. 22, 113-122.

TEXTOR, S., KRAKER, DE, J.-W., HAUSE, B., GERSHENZON, J., TOKUHISA, J.G., 2007: MAM3 catalyzes the formation of all aliphatic glucosinolate chain lengths in Arabidopsis. Plant Phys. 144, 60-71.

WITTSTOCK, U., HALKIER, B.A., 2002: Glucosinolate research in the *Arabidopsis* era. Trends in Plant Science 7, 263-270.

WOLFSON, J.L., 1982: Developmental responses of *Pieris rapae* and *Spodoptera eridania* to environmentally induced variation in *Brassica nigra*. Environ. Entomol. 11, 207-213.

ZHAO, Y., HULL, A.K., GUPTA, N.R., GOSS, K.A., ALONSO, J., ECKER, J.R., NORMANLY, J., CHORY, J., CELENZA J.L., 2002: Trp-dependent auxin biosynthesis in *Arabidopsis*: involvement of cytochrome P450s CYP79B2 and CYP79B3. Genes Developm. 16, 3100-3112.

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