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RESEARCH PAPER

The influence of metabolically engineered glucosinolates profiles in *Arabidopsis thaliana* on *Plutella xylostella* preference and performance

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Abstract The oviposition preference and larval performance of the diamondback moth (DBM), *Plutella xylostella*, was studied using *Arabidopsis thaliana* plants with modified glucosinolate (GS) profiles containing novel GSs as a result of the introduction of individual *CYP79* genes. The insect parameters were determined in a series of bioassays. The GS content of the plants as well as the number of trichomes were measured. Multivariate analysis was used to determine the possible relationships among insect and plant variables. The novel GSs in the tested lines did not appear to have any unequivocal effect on the DBM. Instead, the plant characteristics that affected larval performance and larval preference did not influence oviposition preference. Trichomes did not affect oviposition, but influenced larval parameters negatively. Although the tested *A. thaliana* lines had earlier been shown to influence disease resistance, in this study no clear results were found for *P. xylostella*.

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Introduction

Plants are constantly exposed to stresses that have an impact on their growth. Among these various stresses, insect pests can be very devastating to plant growth and reproduction. To overcome these setbacks, plants have various ways of defending themselves. Glucosinolates (GSs) are known to play a prominent role in plants protecting themselves against pests (Mithen et al. 1995). These amino acid-derived natural products are found in plants of the Brassicaceae and related families, including oilseed rape, cabbage and the model plant *Arabidopsis thaliana* (Halkier and Gershenzon 2006). Upon tissue damage, non-toxic GSs are rapidly hydrolysed to biologically active break-down products by the thioglucosidase myrosinase. Among the hydrolysis products, the defensive function of the glucosinolate–myrosinase system has mainly been attributed to the isothiocyanates that have been shown to be toxic to microorganisms, nematodes and insects. GS biosynthesis occurs in three stages: first, the chain elongation of the precursor amino acid; second, the formation of the core GS structure and; finally, the secondary modifications which include double-bond formation, hydroxylation and methoxylation reactions (Wittstock and Halkier 2002). In the first committed step in the biosynthesis of the core structure of GSs, the precursor amino acid is converted to the corresponding aldoxime. This is a common step in the biosynthesis of GSs and cyanogenic glucosides, another group of amino acid-derived natural products that is widely distributed in the plant kingdom. In the biosynthesis of both GSs and cyanogenic glucosides, aldoxime formation is

catalysed by cytochrome P450 monoxygenases (CYPs) of the CYP79 family. Among the CYP79 homologues that have been overexpressed in Arabidopsis are 'the cyanogenic' CYP79A1 from *Sorghum bicolor* (Poaceae) that converts tyrosine to 4-hydroxyphenylacetaldoxime (Koch et al. 1995), 'the cyanogenic' CYP79D2 from cassava (*Manihot esculenta*, Euphorbiaceae) that converts valine and isoleucine to the respective aldoximes (Andersen et al. 2000), and CYP79A2 from *A. thaliana* that catalyses the conversion of phenylalanine to phenylacetaldoxime (Wittstock and Halkier 2000). The transgenic *A. thaliana* lines overexpressing these CYP79s accumulate high levels of GSs that are not naturally present in *A. thaliana* leaves or only present in minute amounts (Bak et al. 1999; Wittstock and Halkier 2000; Mikkelsen and Halkier 2003). These plants are thought to be a valuable tool to study the impact of GSs with different side-chain structures on insect behaviour and performance.

In addition to these in-built chemical compounds, plants have physical barriers like leaf trichomes, which deter oviposition and insect feeding (Mauricio 1998). Insect behaviour and performance can have strong visible effects depending on the physical barriers and chemical composition of a plant. Hence, resistance can be achieved by manipulating these factors resulting in reduced oviposition and larval feeding. Oviposition preference and offspring performance may vary depending on the larval ability to utilize the host plant (Thompson 1988). Earlier studies have suggested that the accumulation of GSs decreases feeding by generalist herbivores, whereas specialist herbivores have not shown any feeding preference to plants with varying GS levels (Giamoustaris and Mithen 1995; Gigolashvili et al. 2007a, b; Beekwilder et al. 2008; Kliebenstein et al. 2002; Li et al. 2000; Bidart-Bouzat and Kliebenstein 2008; Nielsen et al. 2001). Diamondback moth (DBM), *Plutella xylostella* (L.) is a specialist herbivore known to be a destructive pest of Brassica crops. The DBM is attracted to its host by olfactory, gustatory and tactile stimuli (Badenes-Perez et al. 2004; Bukovinszky et al. 2005). Previous oviposition studies have shown that DBM generally do not lay eggs on non-host plants (Sarfraz et al. 2006). DBM adults are attracted to volatiles emanating from their host plants (Pivnick et al. 1990; Reddy et al. 2004). Both intact GSs and volatile isothiocyanates derived from aliphatic GSs stimulate DBM oviposition when applied to artificial substrates or non-host leaves (Reed et al. 1989; Renwick et al. 2006). DBM larval feeding is not only stimulated by GSs and other secondary metabolites (Nayar and Thorsteinson 1963; Van Loon et al. 2002), but also triggered by nutrients such as sugars, amino acids and primary metabolites that are present on the plant. The larvae are biochemically adapted to the intake of large amounts of GSs and myrosinase. In their gut, they possess a GS sulfatase that converts GSs into desulfoglucosinolates that are not substrates for myrosinases and that are excreted with the faeces (Ratzka et al. 2002).

In the present study, we determined whether the presence of novel GSs in *A. thaliana* has any effect on the oviposition preference and larval performance of the specialist *P. xylostella*. Plants engineered to contain 4-hydroxybenzylglucosinolate, benzylglucosinolate or 1-methylpropylglucosinolate and isopropylglucosinolate were tested.

Materials and methods

Plants

Arabidopsis thaliana of the following genotypes were used: wild-type Columbia-0 (Col-0), 35S:CYP79A1 (lines 1.8 and 6.3.1) producing 4-hydroxybenzylglucosinolate (Bak et al. 1999), 35S:CYP79A2 (lines 30.6 and 10.1) producing benzylglucosinolate (Wittstock and Halkier 2000), 35S:CYP79D2 (lines 28 and 5) producing methyl-ethyl- and isobutylglucosinolate (Mikkelsen and Halkier 2003). Seeds were surface-sterilised for 20 min in 2% sodium hypochlorite followed by a brief rinse with 50% ethanol before planting in sterile soil. The pots were grown in controlled environment of a growth chamber using a 16/8 h photoperiod at 22/18°C.

Insects

Adult females and males and larvae of the DBM (*P. xylostella*) were obtained from the continuous laboratory culture at the Department of Ecology at the Swedish University of Agricultural Sciences reared on cabbage (*Brassica oleracea* ssp. oleracea var. capitata) and *B. napus*. Although the culture has been maintained for many years, new individuals are added each summer.

Bioassays with DBM

Oviposition preference

Adult moths were obtained for the experiment by removing pupae from the laboratory culture. Upon eclosion, adult moths were sexed and pairs of moths (one female and one male) were placed in ventilated containers with access to 10% sugar solution. The experiment was carried out in net cages (80 cm high × 80 cm wide × 40 cm deep), which represented the blocks in a complete block design. In each block (cage) three plants of each of the different lines were placed in a randomly assigned position in a hexagonal grid within the cages. Approximately 6–7 cm inter-plant

distance was maintained. Four-week-old plants were used in the experiment. At the start of each cage, ten pairs of adult moths were released into the cage; we have used this number of moths in an earlier study (Handley et al. 2005) and a comparable number of moths have been used in other studies (Reddy et al. 2004; Sarfraz et al. 2007). DBM females are unaffected by the presence of conspecific eggs (Groeters et al. 1992; Handley et al. 2005). In total, ten blocks (cages) were used for oviposition. On the sixth day after the release of the moths, the plants were removed and the number of eggs laid on each plant was counted; the location of the eggs on the upper or lower leaf surface was noted.

Larval performance in non-choice tests

Newly emerged male and female adult moths were released into cages containing plants from the same lines as the oviposition experimental plants. The moths laid eggs on these plants and they were checked daily for newly hatched larvae. One larva was placed on a new experimental plant of the same genotype as the plant it hatched upon with a paintbrush. Every day the larvae were monitored for the number of days they took to pupation, and after attaining pupation, their weight was recorded. The damaged leaves were photographed using the NIKON SMZ1500 microscope and the percentage leaf area eaten by the larvae was calculated by using the NIS-Elements D 3.00 Imaging software (Nikon). The pupae were collected separately in vials and the number of days to the emergence of the adult and sex were recorded. The experiment was replicated in 20 sets. Each replicate contained all seven lines including the wild-type Col-0.

Larval choice

Third instar larvae were placed in petri dishes (8.5 cm × 1.5 cm) that contained leaves from the seven lines. The leaves were placed in a circle around the edge of the dish in a random order. Prior to feeding, the leaves were photographed using a digital camera and also the microscope (Nikon SMZ1500). Seven larvae were placed in the centre of the dish and allowed to feed on the leaves for 2 h. Every 30 min, data were recorded as to how many larvae were feeding on each leaf. Two hours later, leaf area of the damaged leaves was recorded for further analysis.

Plant characteristics

Trichomes

Trichomes were counted on the leaves from ten plants of all lines grown under the same conditions as those used in the oviposition tests. The number of trichomes was counted within a 20-mm^2 area of both the upper and lower surfaces of the leaf using a NIKON SMZ1400 microscope. Three areas were counted on each leaf. Because there were only a few trichomes present on the lower surface of the leaf, these data were not included for further analyses.

Glucosinolate analysis

Just before the start of the oviposition experiment, 50–100 mg of leaf tissue from each of ten plants from the respective lines was harvested, freeze-dried and analysed for GS content. Preparation of samples for GS determination as desulfoglucosinolates was as described previously (Burow et al. 2006) using 25 µl 1 mM allylglucosinolate per sample as an internal standard. After desulfation, samples were dried at 50°C under a nitrogen stream and redissolved in 200 µl water. Fifty microlitres of the samples was analysed by HPLC on an Agilent HP1200 Series instrument equipped with a C-18 reversed phase column (LiChrospher 100 RP18, 250 × 4.6 mm, 5 µm particle size, Wicom) as described in Burow et al. (2006). Desulfoglucosinolates were identified based on comparison of retention times and UV absorption spectra with those of known standards (Reichelt et al. 2002). The identities of 1-methylpropylglucosinolate and isopropylglucosinolate were confirmed by LC–MS analysis of the desulfoglucosinolates (Mikkelsen and Halkier 2003; see below). GS contents were calculated as described in Burow et al. (2006) using the relative response factors 1.0 for aliphatic GSs, 0.5 for 4-hydroxybenzylglucosinolate, 0.9 for benzylglucosinolate, 0.25 for indole GSs and 0.4 for 3-benzoyloxypropylglucosinolate (Burow et al. 2006; Brown et al. 2003; Haughn et al. 1991; Buchner 1987). For LC–MS analysis, samples were diluted tenfold, and 10 µl was injected into an Agilent HP1200 Series HPLC coupled to a 3200 QTrap MS instrument (Applied Biosystems/MDS Sciex). Separation of the desulfoglucosinolates was achieved on a Lichrospher 100 $C18_{ec}$ column (250 × 2 mm; Goehler Analysentechnik; Germany) using 50 µM sodium acetate (solvent A) and methanol (solvent B) at 0.4 ml/min. The gradient was as follows: 99% A for 3 min, 99–95% A in 5 min, 95–93% A in 2 min, 93–79% A in 10 min, 79–71% A in 5 min, 71–57% A in 7 min. The mass spectrometer equipped with an electrospray ionization interface (ESI, Turbo V) was operated in positive-ion mode and in enhanced product ion (EPI) scan mode. Ionization and EPI conditions were as follows: source voltage 4.5 kV, capillary temperature 400°C, curtain gas at 40 psi, GS1 60 psi, GS2 45 psi, declustering potential 51 V and collision energy 35 V. Nitrogen was used as curtain and auxiliary gas.

Statistical analysis

Insect bioassays

The number of eggs on each plant was subjected to a one-way ANOVA with plant lines as the factor. Differences between lines were tested using a least squares means *t* test. A replicated (with cages as replicates) goodness of fit test using the *G*-statistic (Sokal and Rohlf 1995) was used to determine if the distribution of the eggs on the plants differed from the null hypothesis that oviposition was equally probable on all plants. To determine if the pattern of deposition of eggs on the upper and lower leaf surfaces differed between lines the proportion (using an arsine/square root transformation) of eggs laid on the upper side of the leaves of different lines was analysed using a one-way ANOVA with plant lines as the factor. Larval performance in no-choice tests was subjected to a one-way ANOVA, (factor = plant lines) where the number of leaves consumed and development time were log transformed; pupal weights were normally distributed and were therefore not transformed. Two variables concerning larval choice were analysed using ANOVA (one-way, factor = plant line); (1) percentage of leaf eaten (transformed using arcsine-square root) and (2) number of larvae found on each leaf [transformed using log (number of larvae +1)]. Statistics were carried out in SAS for Windows version 9.1.

Plant characteristics

Trichome data did not differ from a normal distribution and a one-way ANOVA with plant line as the factor was performed. A least squares means test was used to test differences between lines. Total GS contents and proportions of novel GSs (obtained by dividing novel GS content by total GS content) were tested using a one-way ANOVA. Statistics were carried out in SAS for Windows version 9.1. In order to examine the composition of GSs in relation to the investigated plant lines a principle component analysis (PCA) analysis was carried out in CANOCO, version 4.5 (ter Braak and Smilauer 2002). Individual GSs were expressed as a percentage of the total GS content.

Insect responses in relation to plant characteristics

To obtain an overall picture of the relationship between insect responses and the plant characteristics, all data were subjected to a redundancy analysis (RDA) and this was performed using CANOCO, version 4.5 (ter Braak and Smilauer 2002).

Results

Bioassays with *Plutella xylostella*

Oviposition

Ovipositing *P. xylostella* responded differently to the various 35S:CYP79 lines (Table 1). The total number of eggs deposited on the seven plant lines differed significantly among lines ($F = 2.76$, $df = 6,203$, $P = 0.01$). There was no significant difference observed between the number of eggs laid on the wild type and the other plant genotypes. The

Table 1 *Plutella xylostella* (diamondback moth) preference and performance on *A. thaliana* genotypes with different glucosinolate profiles

	Plant genotypes						
	Col-0	CYP79A1.18	CYP79A1.6.3.1.1	CYP79A2.30.6	CYPA2.10.1	CYP79D2.28	CYP79D2.5
Oviposition preference							
Number of eggs	13.5 (2.10) ab	18.1 (1.96) a	9.3 (1.88) b	12.8 (2.21) ab	14.0 (2.17) ab	19.7 (2.74) a	18.7 (2.73) a
Proportion eggs	0.04 (0.004) b	0.06 (0.008) a	0.025 (0.004) c	0.04 (0.004) b	0.05 (0.004) ab	0.06 (0.008) a	0.05 (0.007) ab
Larval performance							
No choice							
Percentage of leaf eaten	3.40 (0.34)	3.75 (0.23)	3.65 (0.31)	2.80 (0.25)	3.10 (0.32)	2.80 (0.26)	2.75 (0.31)
Days to adult	6.8 (0.25)	7.0 (0.15)	6.5 (0.17)	6.7 (0.21)	6.6 (0.16)	6.4 (0.16)	6.5 (0.17)
Pupal weight (mg)	4.24 (0.13)	4.52 (0.13)	4.42 (0.11)	4.61 (0.15)	4.37 (0.20)	4.38 (0.22)	4.36 (0.14)
Choice							
Percentage of leaf eaten	23.8 (4.23) a	20.9 (4.54) a	27.8 (5.00) a	23.5 (2.27) a	9.9 (4.34) b	18.1 (2.74) a	10.9 (4.03) b
Number of larvae on leaf	1.6 (0.34) a	1.2 (0.25) a	1.3 (0.26) a	1.8 (0.20) a	0.6 (0.27) b	1.1 (0.18) a	0.5 (0.17) b

Values in the table are means and standard error (in parentheses). Different letters within a row indicate significant differences ($P < 0.05$), least squares means *t* test

lowest number of eggs was laid on 35S:CYP79A1 line 6.3 and this was significantly different from the number of eggs on 35S:CYP79A1 line 18 and the 35S:CYP79D2 lines. When the distribution of eggs was considered the *G*-statistic for the total (all replicates, $G = 545.59$, $df = 60$, $P < 0.0001$) and the pooled data ($G = 175.63$, $df = 6$, $P < 0.0001$) showed a significant difference from the null hypothesis that eggs were deposited without preference. The *G*-statistic for heterogeneity ($G = 369.96$, $df = 54$, $P < 0.0001$) was also significant and indicated that preference for plant lines was not consistent between replicates (Sokal and Rohlf 1995). The proportion of eggs laid on upper leaf surface did not differ between plant lines ($F = 1.64$, $df = 6,63$, $P = 0.15$). The mean proportion of eggs laid on the upper side of the leaf was 0.58 (standard error 0.02).

Larval performance in no choice tests

Larval performance and feeding behaviour were analysed by measuring the leaf area consumed, the number of days to adult emergence and weight of the pupae (Table 1). No significant differences among the lines were found for any of the three measurements.

Larval choice

There was a significant difference for leaf area eaten ($F = 2.83$, $df = 6,63$, $P = 0.02$) and larvae on the leaves ($F = 3.73$, $df = 6,63$, $P = 0.004$). One of the 35S:CYP79A2 lines (line 10.1) and one of the 35S:CYP79D2 lines (line 5) were significantly different from the wild type and all other genotypes in terms of leaf area removed and number of larvae found on the plants (Table 1). They were the two lines from which the least amount of leaf was eaten and on which the lowest number of larvae was found (Table 1). The two 35S:CYP79A1 lines did not differ from each other or from the wild-type.

Plant characteristics

Trichomes

The number of trichomes differed significantly among lines (Table 2). There was no significant difference between the numbers of trichomes on the 35S:CYP79 lines (Table 2). The Col-0 wild type, which had the lowest mean number of trichomes, was only significantly different from the 35S:CYP79D2 line 28 (Table 2).

Glucosinolates

Total GS content (nmol/mg dry weight) differed among genotypes as did the content of most of the individual GSs (Table 2). The GS composition (Fig. 1) showed clustering among the lines of the three 35S:CYP79 mutants. As expected 35S:CYP79A1 line 18 and line 6.3 contained 4-hydroxybenzylglucosinolate, and benzylglucosinolate was found only in 35S:CYPA2 line 10.1 and line 30.6 (Table 2; Fig. 1). Unexpectedly, detectable amounts of isopropyl- and 1-methylpropylglucosinolate were only found in 35S:CYP79D2 line 28, but not in line 5 (Table 2). The content of benzylglucosinolate in 35S:CYP79A2 plants was positively associated with the content of 8-methylsulfinyloctylglucosinolate, and the contents of isopropyl- and 1-methylpropylglucosinolates in 35S:CYP79D2 plants were positively associated with the content of 7-methylsulfinylheptylglucosinolate (Fig. 1, arrows in the same direction).

Insect responses in relation to plant characteristics

In the multivariate analysis of all the variables, the RDA shows that most of the plant characteristics (red text in Fig. 2) line up with (positive correlation) or are opposite to (negative correlation) the larval performance and larval choice variables. For example, high proportions of 1-methoxyindol-3-ylmethylglucosinolate, 3-benzoyloxypropylglucosinolate or 4-hydroxybenzylglucosinolate were associated with higher larval preference and an increased pupal weight. However, they were also associated with prolonged larval development. Several GSs with methylsulfinylalkyl-side chains as well as the number of trichomes had the opposite effects. The plant characteristics that affected larval performance and larval preference did not influence oviposition preference (total eggs and proportion of eggs). The proportion of 7-methylsulfinylheptylglucosinolate was positively correlated with oviposition preference. For several parameters in our analysis (proportion of benzylglucosinolate, indol-3-ylmethylglucosinolate, 4-methoxyindol-3ylmethylglucosinolate, total GS content), the length of the arrows was so short that one cannot assume any clear influence.

Discussion

In the present study, DBM did not appear to be influenced by total GS content. On the other hand, there was evidence that larval performance and feeding choice were influenced by GSs (Fig. 2). Insect responses to *A. thaliana* with metabolically engineered GS profiles were, however, not governed by the novel GSs in any simple manner. It does not appear that individual GSs will change plant acceptance or resistance for DBM. Among the CYP79A1 lines, where CYP79A1.18 has a higher total content of GSs than CYP79A1.6.3.1.1, CYP79A1.18 had more eggs than its

Table 2 Plant characteristics of *A. thaliana* genotypes with different glucosinolate profiles

	Plant genotypes						
	Col-0	CYP79A1.18	CYP79A1.6.3.1.1	CYP79A2.30.6	CYPA2.10.1	CYP79D2.28	CYP79D2.5
Trichomes							
Number per 20 mm^2 $F = 3.70$, $P = 0.003$	11.0 (0.49) b	12.2 (0.94) ab	13.8 (0.63) ab	13.4 (0.76) ab	14.2 (0.96) ab	15.5 (0.99) a	12.4 (0.37) ab
Glucosinolates nmol/mg dry weight							
Total glucosinolates F = 4.70, p = 0.0005	11.57 (3.15) ab	5.79 (1.05) bc	2.92 (0.90) c	12.93 (1.51) a	7.97 (1.31) abc	8.03 (1.07) abc	5.75 (1.03) bc
p-Hydroxybenzyl- $F = 9.12$, $P < 0.0001$	0 b	1.28 (0.28) a *21%*	0.89 (0.38) a *22%*	0 b	0 b	0 b	0 b
Benzyl- $F = 59.3$, $P < 0.0001$	0 c	0 c	0 c	4.85 (0.50) a *39%*	2.16 (0.40) b *26%*	0 c	0 c
Methylethyl- $F = 15.4$, $P < 0.0001$	0 b	0 b	0 b	0 b	0 b	0.65 (0.17) a *8%*	0 b
Isobutyl- $F = 10.8$, $P < 0.0001$	0 b	0 b	0 b	0 b	0 b	0.24 (0.07) a *3%*	0 b
3-Methylsulfinylpropyl- $F = 3.36$, $P = 0.006$	0.97 (0.30) a *8%*	0.37 (0.07) b *7%*	0.19 (0.05) b *7%*	0.66 (0.08) ab *5%*	0.52 (0.07) ab *7%*	0.60 (0.08) ab *7%*	0.53 (0.10) ab *9%*
4-Methylsulfinylbutyl- $F = 3.76$, $P = 0.003$	8.40 (2.49) a *68%*	3.46 (0.65) b *60%*	1.53 (0.36) b *62%*	5.48 (0.72) ab *41%*	4.20 (0.67) b *54%*	5.64 (0.77) ab *69%*	3.87 (0.54) b *72%*
7-Methylsulfinylheptyl- $F = 3.84$, $P = 0.003$	0.18 (0.05) a *1%*	0.08 (0.01) ab *1%*	0.03 (0.02) b	0.14 (0.03) a *1%*	0.10 (0.01) ab *1%*	0.17 (0.03) a *2%*	0.08 (0.02) ab *1%*
Indol-3-ylmethyl- $F = 16.2$, $P < 0.0001$	1.63 (0.30) a *18%*	0.48 (0.08) b *9%*	0.20 (0.09) b *5%*	0.27 (0.03) b *2%*	0.14 (0.05) b *2%*	0.64 (0.07) b *10%*	0.38 (0.04) b *8%*
8-Methylsulfinyloctyl- $F = 11.33$, $P < 0.0001$	0 c	0 c	0.03 (0.03) c *1%*	1.50 (0.19) a *12%*	0.82 (0.14) b *10%*	0 c	0.79 (0.40) b *8%*
4-Methoxyindol-3-ylmethyl- $F = 65.3$, $P < 0.0001$	0.30 (0.03) a *4%*	0.07 (0.01) bc *1%*	0.04 (0.01) cd *2%*	0.02 (0.01) d	0.004 (0.004) d	0.10 (0.01) b *1%*	0.09 (0.01) b *2%*
3-Benzoyloxypropyl- $F = 2.04$, $P = 0.07$	0.03 (0.02)	0.01 (0.01)	0	0	0	0	0
1-Methoxyindol-3-ylmethyl- $F = 4.12$, $P = 0.002$	0.07 (0.02) a *1%*	0.05 (0.004) ab *1%*	0.03 (0.02) ab	0.01 (0.01) b	0.03 (0.01) ab	0.003 (0.003) b	0.004 (0.004) b

Values in the table are means and standard error (in parentheses). Results of the ANOVA are shown with the plant characteristic. Degrees of freedom for all *F* tests are *df* 6,63. Different letters within a row indicate significant differences ($P < 0.05$), Student–Newman–Keuls test. For individual glucosinolates per cent of total glucosinolates is shown in italics style (percentages <1% not shown)

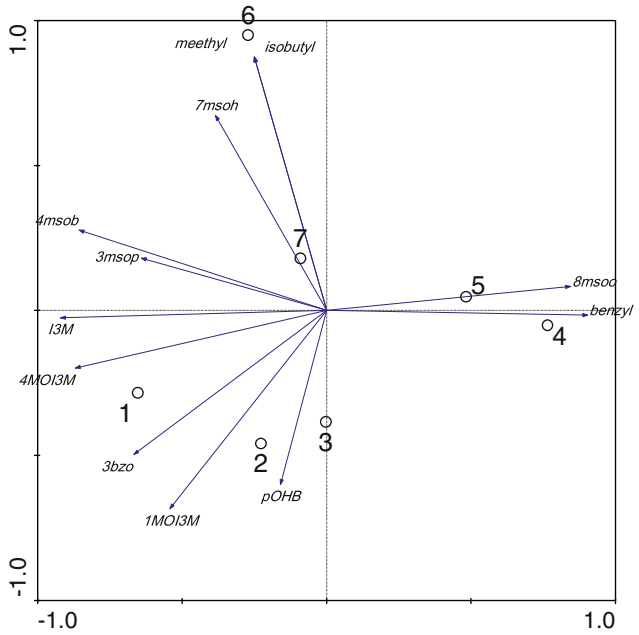

Fig. 1 Glucosinolate composition of the different 35S:CYP79 transgenic lines. PCA using proportion of total glucosinolates. Eigenvalues are 0.443 for axis 1 and 0.268 for axis 2. The two axes explain 71.1% of the variation. Plant genotypes are represented by *circles* and are as follows: *1* Col-0, *2* 35S:CYP79A1.18, *3* 35S:CYP79A1.6.3.1.1, *4* 35S:CYP79A2.30.6, *5* 35S:CYPA2.10.1, *6* 35S:CYP79D2.28, *7* 35S:CYP79D2.5. Glucosinolates (*GS*) are: *3msop* 3-methylsulfinylpropyl-GS, *4msob* 4-methylsulfinylbutyl-GS, *7msoh* 7-methylsulfinylheptyl-GS, *I3M* indol-3-ylmethyl-GS, *4MOI3M* 4-methoxyindol-3-ylmethyl-GS, *3bzo* 3-benzoyloxypropyl-GS, *1MOI3M* 1-methoxyindol-3-ylmethyl-GS, *isobutyl* isobutyl-GS, *meethyl* methylethyl-GS, *benzyl* benzyl-GS, *8msoo* 8-methylsulfinyloctyl-GS, *pOHB* 4-hydroxybenzyl-GS

counterpart CYP79A1.6.3.1.1 (Table 1). There were, unfortunately, no clear patterns for oviposition preference. The moths showed preferences as indicated by the significant *G*-statistics for total and pooled data, but the preferences were not consistent as shown by the significant *G*-statistic for heterogeneity. The two replicate lines of each type showed variation in total GS amounts and in GS composition and this may have made it more difficult to detect trends. The differences suggest that the position of the transgene resulted in different expression levels of the transgene. This is expected to result in production of different amounts of end product. The variation in GS profile amongst transgenic lines of the same transgene suggests that the pull on a given amino acid pool may influence the pool size of other amino acids and thereby the precursor availability and production of other GSs. Alternatively, the sulphur availability may be limiting, and therefore the sulphur will be directed towards different GS dependent on 'glucosinolate sink strength'.

Previous studies have revealed that DBM is dependent upon the host plant for the onset of egg production (Åsman and Ekbom 2006). A few GSs such as allylglucosinolate

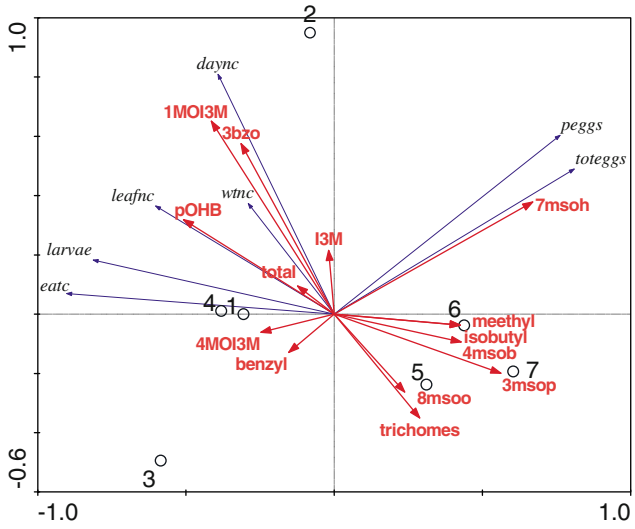

Fig. 2 Analysis of insect preference and performance together with plant characteristics. RDA, variables are centred and standardized. Eigenvalues are 0.473 for axis 1 and 0.224 for axis 2. The two axes explain 69.7% of the variation. Plant genotypes are represented by *circles* and are as follows: *1* Col-0, *2* 35S:CYP79A1.18, *3* 35S:CYP79A1.6.3.1.1, *4* 35S:CYP79A2.30.6, *5* 35S:CYPA2.10.1, *6* 35S:CYP79D2.28, *7* 35S:CYP79D2.5. Values in *red* are plant characteristics: glucosinolates as in Fig. 1 and trichomes. Response variables are: *eatc* % leaf area eaten in choice test, *larvae* number of larvae found on a genotype (choice test), *leafnc* % leaf area eaten in no-choice test, *wtnc* final pupal weight in no-choice test, *daync* number of days to pupation in no-choice test, *pegg* % of eggs laid on a genotype, *totegg* total eggs laid on a genotype

and indol-3-ylmethylglucosinolate are known to act as stimulants for oviposition of DBM (Talekar and Shelton 1993; Shelton 2004). But proportions of indol-3-ylmethylglucosinolate did not appear to influence oviposition while 7-methylsulfinylheptylglucosinolate did. It has also been reported that certain glucosinolates with different side chains, for example, benzylglucosinolate, do not act as an oviposition stimulant (Renwick and Radke 1990), and in our analysis benzylglucosinolate did not appear to affect oviposition (Fig. 2). There are reports that oviposition may also depend on the pattern of wax on the leaf surface (Chen et al. 2003). The level of an individual GS might be less important for insect responses than the combination and levels of several GSs.

Even though the trichome density on the Col-0 wild type differed from one of the 35S:CYP79 lines (35S:CYP79D2 line 28), this did not have any effect on oviposition pattern (Fig. 2). The 35S:CYP79A1 lines grew more slowly and were smaller than the wild type, but this did not appear to affect trichome density or oviposition pattern. This is in contrast to a previous study by Handley et al. (2005), which reported an increased resistance to DBM oviposition among the *A. thaliana* populations with higher numbers of trichomes (variation was between about 15–25 trichomes). It may be, however, that variation in trichome number

among genotypes in the present study (11–15.5) was not high enough to detect oviposition differences. More trichomes appear to be associated with less leaf area eaten and larval performance parameters in our study, but Handley et al. (2005) found no significant relationship between larval performance measures and trichome density.

In the no-choice test, where the larvae were allowed to feed on one of the 35S:CYP79 lines or the wild-type, the different genotypes did not have a significant effect on the number of days to adult emergence or on pupal mass (Table 1). Pupae did not show any signs of mortality either. Since DBM is a specialist on Brassicaceae and DBM larvae possess a highly efficient detoxification system for GSs (Ratzka et al. 2002), this result may be expected. Larvae seem to be well adapted to plants with high GS content. This is in agreement with a previous study in which feeding on *B. juncea* lines with high-GS content did not affect DBM larvae (Li et al. 2000). Arany et al. (2008) observed no difference in DBM performance on *A. thaliana* expressing higher levels of GSs but found a negative correlation in the generalist *Spodoptera exigua*. In a common garden experiment, Bidart-Bouzat and Kliebenstein (2008) observed that a specialist insect like DBM was found to feed heavily on *A. thaliana* plants with higher GS content. They also reported that individual GSs of both the aliphatic and indole types as well as total aliphatic and indole GS content were positively associated with high insect damage.

When given a choice, DBM larvae seemed to prefer some genotypes over others (Table 1). The least number of larvae were found on the 35S:CYP79A2.10.1 and 35S:CYP79D2.5 leaves. These two genotypes, however, have no apparent relationship to each other concerning their GS profile (Fig. 1). Crucifer-specialist flea beetles (*Phyllotreta nemorum* and *P. cruciferae*) did not show any preference in feeding between CYP79A1 and wild type (Col-0) plants (Nielsen et al. 2001). These authors concluded that the response to GSs would be dependent on the chemical and physical environment which complicates the picture considerably.

Previous work by Brader et al. (2006) using the same genotypes as those used in this study (35S:CYP79A1, 35S:CYP79A2 and 35S:CYP79D2 plants) has shown a differential disease resistance pattern against pathogens. 35S:CYP79A1 and 35S:CYP79A2 plants had higher susceptibility towards the necrotrophic fungal pathogen *Alternaria brassicicola*, whereas 35S:CYP79D2 plants were more resistant. 35S:CYP79A1 and 35S:CYP79A2 lines were resistant to the hemibiotroph *Pseudomonas syringae*. Plant pathogens living directly on the plant may be more sensitive to plant chemical composition than a specialist insect like DBM.

With this study, we show that *A. thaliana* plants that ectopically express CYP79 enzymes catalysing aldoxime formation in the biosynthesis of GSs and therefore accumulate additional GSs have only a limited value for studying the signals mediating the interaction of DBM larvae and adults with their host plants. This interaction seems to be very complicated and likely also involves other signals than just the GSs (van Loon et al. 2002). Furthermore, individual GSs do not appear to govern the preference and performance of DBM.

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