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.

Keywords CYP79 · Diamondback moth · Trichomes · Oviposition

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 breakdown 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 Arabidopsis 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; Gogolashvili et al. 2007a, b; Beekwilder et al. 2008; Kliebenstein et al. 2002; Li et al. 2000; Bidart-Bouzet 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 Arabidopsis 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 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-mm² 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/MD Sciex). Separation of the desulfoglucosinolates was achieved on a Lichrospher 100 C18ec 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 arcsine/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.

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

| Plant genotypes | Col-0 | CYP79A1.18 | CYP79A1.6.3.1.1 | CYP79A2.30.6 | CYP9A2.10.1 | 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        | 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) |
| Percentage of leaf eaten | 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) |
| Days to adult    | 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) |
| Pupal weight (mg) |      |           |                |             |             |            |           |
| Choice           | 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 |
| Percentage of leaf eaten | 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.

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.
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:CYP79A2 line 10.1 and line 30.6 (Table 2; Fig. 1). Unexpectedly, detectable amounts of isopropyl- and 1-methylpropylglucosinolates 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-methylsulfinylheptylglucosinolate, 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) 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-methylsulfanylheptylglucosinolate was positively correlated with oviposition preference. For several parameters in our analysis (proportion of benzylglucosinolate, indol-3-ylmethylglucosinolate, 4-methoxyindol-3-ylmethylglucosinolate, 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 | CYP79A1.18 | CYP79A1.6.3.1.1 | CYP79A2.30.6 | CYP79A2.10.1 | 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 | 13.6 (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- | $F = 9.12, P < 0.0001$ | 0.00 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 | 0 b |
| Isobutyl- | $F = 10.8, P < 0.0001$ | 0 b | 0 b | 0 b | 0 b | 0 b | 0.24 (0.07) a | 0 b |
| 3-Methylsulfinylpropyl- | $F = 3.36, P = 0.006$ | 0.97 (0.30) a | 0.37 (0.07) b 7% | 0.19 (0.05) b | 0.66 (0.08) ab | 0.52 (0.07) ab | 0.60 (0.08) ab | 0.53 (0.10) ab |
| 4-Methoxyindol-3-ylmethyl- | $F = 65.3, P < 0.0001$ | 0.30 (0.05) a | 0.08 (0.01) ab 1% | 0.03 (0.02) b 1% | 0.14 (0.03) a 1% | 0.10 (0.01) ab 1% | 0.17 (0.03) a | 0.08 (0.02) ab 1% |
| 3-Benzoyloxypropyl- | $F = 2.04, P = 0.07$ | 0.03 (0.02) b 1% | 0.01 (0.01) cd 2% | 0.04 (0.01) cd 2% | 0.02 (0.01) d 1% | 0.004 (0.004) d 1% | 0.01 (0.01) b 1% | 0.09 (0.01) b 1% |
| 1-Methoxyindol-3-ylmethyl- | $F = 4.12, P = 0.002$ | 0.07 (0.02) a | 0.05 (0.04) ab 1% | 0.03 (0.02) ab 1% | 0.03 (0.02) ab 1% | 0.03 (0.01) ab 1% | 0.003 (0.003) b 1% | 0.004 (0.004) b 1% |

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).
and indol-3-ylmethylglucosinolate are known to act as oviposition stimulants for DBM (Talekar and Shelton 1993; Shelton 2004). But proportions of indol-3-ylmethylglucosinolate did not appear to influence oviposition while 7-methyisulfinylethylglucosinolate 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 hemibiotrophic 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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**References**

Andersen MD, Busk PK, Svendsen I, Møller BL (2000) Cytochromes P-450 from cassava (Manihot esculenta Crantz) catalyzing the first steps in the biosynthesis of the cyanogenic glucosides linamarin and lotaustralin—cloning, functional expression in Pichia pastoris, and substrate specificity of the isolated recombinant enzymes. J Biol Chem 275:1966–1975

Arany AM, de Jong TJ, Kim HK, van Dam NM, Choi YH, Verpoorte R, van der Meijden E (2008) Glucosinolates and other metabolites in the leaves of Arabidopsis thaliana from natural populations and their effects on a generalist and a specialist herbivore. Chemoecology 18:65–71

Åsman K, Ekbom B (2006) Responses of ovipositing moths to host plant deprivation: life history aspects and implications for cropping. Agric For Entomol 8:213–219

Badenes-Perez FR, Shelton AM, Nault BA (2004) Evaluating trap crops for diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). J Econ Entomol 97:1365–1372

Bak S, Olsen CE, Petersen BL, Møller BL, Halkier BA (1999) Metabolic engineering of p-hydroxybenzylglucosinolate in Arabidopsis by expression of the cyanogenic CYP79A1 from Sorghum bicolor. Plant J 20:663–671

Beekwilder J, van Leeuwen W, van Dam NM, Bertossi M, Grandi V, Mizzi L, Soloviev M, Szabados L, Molthoff JW, Schipper B, Verbocht H, de Vos RCH, Morandini P, Aarts MGM, Boyv A (2008) The impact of the absence of aliphatic glucosinolates on insect herbivory in Arabidopsis. PLoS One 3(4):e2068. doi: 10.1371/journal.pone.0002068

Bidart-Bouzet MG, Kliebenstein DJ (2008) Differential levels of insect herbivory in the field associated with genotypic variation in glucosinolates in Arabidopsis thaliana. J Chem Ecol 34:1026–1037

Brader G, Mikkelsen MD, Halkier BA, Palva ET (2006) Altering glucosinolate profiles modulates disease resistance in plants. Plant J 46:758–767

Brown PD, Tokuhisa JG, Reichelt M, Gershenson J (2003) Variation of glucosinolate accumulation among different organs and developmental stages of Arabidopsis thaliana. Phytochemistry 62:471–481
Buchner R (1987) Approach to determination of HPLC response factors for glucosinolates. In: Wathieu J-P (ed) Glucosinolates in rapeseeds: analytical aspects. Martinus Nijhoff Publishers, Boston, pp 50–58

Bukovinszky T, Potting RJP, Clough Y, van Lenteren JC, Vet LEM (2005) The role of pre and post-alighting detection mechanisms in the responses to patch size by specialist herbivores. Oikos 109:435–446

Burow M, Müller R, Gershenzon J, Wittstock U (2006) Altered glucosinolate hydrolysis in genetically engineered Arabidopsis thaliana and its influence on the larval development of Spodoptera littoralis. J Chem Ecol 32:2333–2349

Chen S, Glawischning E, Jorgensen K, Naar P, Jorgensen B, Olsen CE, Hansen CH, Rasmussen H, Pickett J, Halkier BA (2003) CYP79F1 and CYP79F2 have distinct functions in the biosynthesis of aliphatic glucosinolates in Arabidopsis. Plant J 5:923–937

Giamoustaris A, Mithen R (1995) The effect of modifying the glucosinolate content of leaves of oilseed rape (Brassica napus ssp. Oleifera) on its interaction with specialist and generalist pests. Ann Appl Biol 126:347–363

Gigolashvili T, Berger B, Mock H-P, Müller C, Flügge U-I (2007a) The transcription factor HIG1/MYB51 regulates glucosinolate hydrolysis in genetically engineered Arabidopsis thaliana. Plant J 50:886–901

Gigolashvili T, Yatusевич R, Berger B, Müller C, Flügge U-I (2007b) The R2R3-MYB transcription factor HAG1/MBY28 is a regulator of methionine-derived glucosinolate biosynthesis in Arabidopsis thaliana. Plant J 51:247–261

Groeters FR, Tabashnik BE, Finson N, Johnson MW (1992) Oviposition preference of the diamondback moth unaffected by the presence of conspecific eggs or Bacillus thuringiensis. J Chem Ecol 18:2353–2362

Halkier BA, Gershenzon J (2006) Biochemistry of plant secondary metabolites in Arabidopsis thaliana. Genetics 161:325–332

Handley R, Ekborn B, Agren J (2005) Variation in trichome density and resistance against a specialist insect herbivore in natural populations of Arabidopsis thaliana. Ecoul Entomol 30:284–292

Haughn GW, Davin L, Giblin M, Underhill EW (1991) Biochemical genetics of plant secondary metabolites in Arabidopsis thaliana. Plant Physiol 97:217–226

Kliebenstein DJ, Peterson D, Barker B, Mitchell-Olds T (2002) Comparative analysis of quantitative trait loci controlling glucosinolates, myrosinase and insect resistance in Arabidopsis thaliana. Genetics 161:325–332

Koch BM, Sibbesen O, Halkier BA, Svendsen I, Møller BL (1995) The primary sequence of cytochrome P450TYR, the multifunctional N-hydroxylase catalyzing the conversion of α-tytosine to P-hydroxyphenylacetaldehyde oxime in the biosynthesis of the cyanogenic glucoside dhurrin in Sorghum bicolor. Arch Biochem Biophys 323:177–186

Li Q, Eigenbrode SD, Stringam GR, Thiagarajah MR (2000) Feeding and growth of Plutella xylostella and Spodoptera eridania on Brassica juncea with varying glucosinolate concentrations and myrosinase activities. J Chem Ecol 26:2401–2419

Mauricio R (1998) Costs of resistance to natural enemies in field populations of the annual plant Arabidopsis thaliana. Am Nat 151:20–28

Mikkelsen MD, Halkier BA (2003) Metabolic engineering of valine and isoleucine derived glucosinolates in Arabidopsis expressing CYP79D2 from Cassava. Plant Physiol 131:773–779

Mithen R, Raybould AF, Giamoustaris A (1995) Divergent selection for secondary metabolites between wild populations of Brassica oleracea and its implications for plant-herbivore interactions. Heredity 75:472–484

Nayar JK, Thorsteinsson AJ (1963) Further investigations into chemical basis of insect-host plant relationships in an oligophagous insect, Plutella maculipennis. Can J Zool 41:923–929

Nielsen JK, Hansen MD, Agerbirk N, Petersen BL, Halkier BA (2001) Responses of the flea beetles Phyllotreta nemorum and P. cruciferae to metabolically engineered Arabidopsis thaliana with an altered glucosinolate profile. Chemoecology 11:75–83

Pivnick KA, Jarvis BJ, Gillot C, Slater GP, Underhill EW (1990) Daily patterns of reproductive activity and the influence of adult density and exposure to host plants on reproduction in the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). Environ Entomol 19:587–593

Ratzka A, Vogel H, Kliebenstein DJ, Mitchell-Olds T, Kroymann J (2002) Disarming the mustard oil bomb. PNAS 99:11223–11228

Reddy GVP, Tabone E, Smith MT (2004) Mediation of host selection and oviposition behavior in the diamondback moth Plutella xylostella and its predator Chrysoperla carnea by chemical cues from cole crops. Biol Control 29:270–277

Reed DW, Pivnick KA, Underhill EW (1989) Identification of chemical oviposition stimulants for the diamondback moth, Plutella xylostella, present in 3 species of Brassicaceae. Entomol Exp Appl 53:277–286

Reichelt M, Brown PD, Schneider B, Oldham NJ, Stauber E, Tokuhisa J, Kliebenstein DJ, Mitchell-Olds T, Gershenzon J (2002) Benzoic acid glucosinolate esters and other glucosinolates from Arabidopsis thaliana. Phytochemistry 59:663–671

Renwick JAA, Radke CD (1990) Plant constituents mediating oviposition by the diamondback moth, Plutella xylostella (L.) (Lepidoptera: Pluttellidae). Physophaga 3:37–46

Renwick JAA, Haribal M, Gouinguene S, Stadler E (2006) Isothiocyanates stimulating oviposition by the diamondback moth, Plutella xylostella. J Chem Ecol 32:755–766

Sarfraz M, Dosdall LM, Keddie BA (2006) Diamondback moth host plant interactions: implications for pest management. Crop Prot 25:625–639

Sarfraz M, Dosdall LM, Keddie BA (2007) Resistance of some cultivated Brassicaceae to infestations by Plutella xylostella. J Econ Entomol 100:215–224

Shelton AM (2004) Management of the diamondback moth: déjà vu all over again? In: Endersby NM, Rildand PM (eds) The management of diamondback moth and other crucifer pests proceedings of the fourth international workshop, 26–29 November 2001. Department of National Resources and Environment, Melbourne, Australia, pp 3–8

Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research, 3rd edn. W.H. Freeman & Company, New York

Talekar NS, Shelton AM (1993) Biology, ecology, and management of the diamondback moth. Annu Rev Entomol 38:275–301
ter Braak CJF, Smilauer P (2002) CANOCO reference manual and environment, Melbourne, Australia, pp 3–8

Thompson JN (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring. Entomol Exp Appl 47:3–14

Van Loon JJA, Wang CA, Nielsen JK, Gols R, Qiu YT (2002) Flavonoids from cabbage are feeding stimulants for diamondback moth larvae additional to glucosinolates: chemoreception and behaviour. Entomol Exp Appl 104:27–34

Wittstock U, Halkier BA (2000) Cytochrome P450 CYP79A2 from Arabidopsis thaliana L. catalyses the expression of L-phenylalanine to phenylacetaldoxime in the biosynthesis of benzylglucosinolate. J Biol Chem 275:14659–14666

Wittstock U, Halkier BA (2002) Glucosinolate research in the Arabidopsis era. Trends Plant Sci 7:263–270