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Performance of specialist and generalist herbivores feeding on cabbage cultivars is not explained by glucosinolate profiles

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Abstract

Plants display a wide range of chemical defences that may differ in effectiveness against generalist and specialist insect herbivores. Host plant-specific secondary chemicals such as glucosinolates (GS) in Brassicaceae typically reduce the performance of generalist herbivores, whereas specialists have adaptations to detoxify these compounds. The concentration of glucosinolates may also alter upon herbivory, allowing the plant to tailor its response to specifically affect the performance of the attacking herbivore. We studied the performance of three Lepidoptera species, two specialists [*Pieris rapae* L. (Pieridae), *Plutella xylostella* L. (Yponomeutidae)] and one generalist [*Mamestra brassicae* L. (Noctuidae)], when feeding on eight cultivars of *Brassica oleracea* L. and a native congener (*Brassica nigra* L.) and related this to the GS content. We tested the hypotheses (i) that a generalist herbivore is more affected by high GS concentrations, and (ii) that generalist feeding has a stronger effect on GS levels. Although performance of the three herbivores was different on the *B. oleracea* cultivars, *M. brassicae* and *P. xylostella* had a similar ranking order of performance on the eight cultivars. In most of the cultivars, the concentration of indole GS was significantly higher after feeding by *P. rapae* or *M. brassicae* than after *P. xylostella* feeding. As a consequence, the total concentration of GS in the cultivars showed a different ranking order for each herbivore species. The generalist *M. brassicae* performed equally well as the specialist *P. xylostella* on cultivars with high concentrations of GS. Our findings suggest that secondary metabolites other than GSs or differences in nutrient levels affect performance of the species studied.

Introduction

Within the plant kingdom, a wide range of morphological and chemical defences against insect herbivory has evolved (Zangerl & Berenbaum, 1990; Cipollini et al., 2003). As a result of frequency-dependent selection by a community of attackers that is highly variable in time and space and shows variable susceptibility to plant defences, the variation in defence expression is often maintained within a single plant species (Mithen et al., 1995; Nielsen, 1997; van Leur et al., 2006). Each attacker may select for a specific set of defence traits in the plant and each expressed defence trait may have different effects on herbivores that differ in their susceptibility to particular defence chemicals (Jaenike, 1990; van der Meijden, 1996).

In brassicaceous plants, the characteristic secondary chemicals, glucosinolates (GS) (Fahey et al., 2001), and their breakdown products are well known to effectively decrease performance of generalist herbivores (Chew, 1988; Olsson & Jonasson, 1994; Traw & Dawson, 2002; Agrawal & Kurashige, 2003). The same chemicals can also reduce performance of specialist herbivores (Agrawal, 2000; van Dam et al., 2000), even though such chemicals attract these specialists or stimulate them to feed (David & Gardiner, 1966; van Loon et al., 1992, 2002; Renwick, 2002). These specialists, however, may be able to neutralize

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the defences present in their host plants. For example, toxic compounds may be rapidly excreted, hydrolyzed, or enzymatically disarmed (Ratzka et al., 2002; Wittstock et al., 2004). Some specialist herbivores even sequester plant defence compounds for their own defence (Schoonhoven et al., 2005; Després et al., 2007) and may be highly tolerant to these compounds (Müller & Sieling, 2006).

Moreover, the concentration of foliar GS has been found to change after herbivory in various *Brassica* species (Griffith et al., 1994; Siemens & Mitchell-Olds, 1998; van Dam et al., 2004). These induced responses of plants (Karban & Myers, 1989) may have evolved as an adaptive trait to reduce the costs of defence when herbivores are absent, and may also enable plants to respond specifically to the type of attacker (Karban & Baldwin, 1997; Zangerl, 2003). Plants may recognize the herbivore that is feeding, based on the pattern of imposed damage and the salivary constituents, creating an opportunity for a fine-tuned response by the plant (Mattiacci et al., 1995; Dicke, 1999; Kahl et al., 2000). Consequently, it may be expected that specialists and generalists have different effects on plant responses. Differential responses by plants to different herbivore species have been found in Brassicaceae (Agrawal, 2000; Traw & Dawson, 2002; Mewis et al., 2006). However, induced plant responses did not always result in induced resistance against the inducing herbivore itself and generalists were not more susceptible than specialists to induced responses of the plant (Agrawal, 2000). Furthermore, plants have also been found to respond similarly to generalist and specialist herbivores (Reymond et al., 2004). There is limited information on the nature of differential responses of plants to herbivory that also result in enhanced resistance against the attacker.

Here, we studied the relationship between specificity in defence pattern expression and the level of specialization of the attacking herbivore. We tested the effect of GS composition and concentration of brassicaceous plants on the performance of three Lepidoptera species and tested whether the host plant specialization level of the herbivores correlates with GS concentrations before and after herbivore attack. *Pieris rapae* L. (Pieridae) and *Plutella xylostella* L. (Yponomeutidae) were studied to represent specialist species; both have overlapping host plant ranges within the Brassicaceae. *Mamestra brassicae* L. (Noctuidae) was used as a generalist, having a host plant range extending beyond the Brassicaceae. We quantified constitutive concentrations of GS in eight different cultivars of *Brassica oleracea* L. and a native congener (*Brassica nigra* L.) and measured GS concentrations after herbivory by caterpillars of the three species. Both within and between wild species as well as crops, there is a vast diversity of GS structures and concentrations (Benrey et al., 1998; Kliebenstein et al., 2002), which differ in their effectiveness as defence compounds (Fahey et al., 2001). The eight cultivars were all selected from the *alba* group to reduce morphological variation among cultivars, and we selected a mix of open pollinated cultivars and more recently cultivated F_1 hybrids from different plant breeders to enhance variation in chemical composition between cultivars. We used *B. nigra* as a reference species, representing a species that has a high level of direct defence with total GS concentrations 3–5-fold higher than found in cultivated plants (Mithen et al., 1995).

We specifically tested the hypothesis that performance of the generalist will be better on the *Brassica* species and cultivars containing low GS concentrations, while we expected no effect on the specialists, predicting that the two specialists are more similar to each other than to the generalist in their rank order of performance. We investigated (i) whether performance of the three herbivore species correlates with the amount of GSs, and (ii) whether a particular herbivore affects a particular set of GS consistently in different cultivars of a single plant species. The implications of our results for the concept of specificity of plant–herbivore interactions are evaluated.

Materials and methods

The following eight cultivars of white cabbage (*B. oleracea* variety *alba*) were used (sources are given between brackets): Domia (Horticulture Research International, Warwick, UK); Badger Shipper, Jersey Queen, and Christmas Drumhead (Centre for Genetic Resources, CGN, Wageningen, The Netherlands), representing open pollinated cultivars, and Lennox, Rivera, and Bartolo (Bejo Zaden BV, Warmenhuizen, The Netherlands) and Stonehead (Sakata Holland BV, Rijsenhout, The Netherlands), representing more recently cultivated F_1 hybrids. Seeds of *B. nigra* were collected in 2000 from a population near Heteren, The Netherlands (51°57′N, 5°45′E), and propagated by open pollination several times since. All plants were grown in a climatized glasshouse compartment. Seeds were germinated (15 November 2004) on peat soil (Lentse potgrond, no. 4; Lent, The Netherlands) and 10 days later individual seedlings were transferred to peat soil in 1.45-l pots. Plants were provided with SON-T light (500 µmol m^{-2} s^{-1}; L16:D8), 18–26 °C, and 50–70% r.h. When the plants were 4 weeks old, they were fertilized by applying Kristalon Blauw (Hydro Agri, Rotterdam, The Netherlands) (N-P-K) 19-6-20-3 micro (2.5 mg l^{-1}) to the soil and this was repeated once every other week during the experiment. Pots with 8-week-old plants were placed individually in trays containing a water layer of 1 cm at the start of the experiment and this water level was maintained during the experiment (see below).

On 10 January 2005 (t = 0), the newest fully grown leaf of 15 plants of each cultivar was sampled for GS analysis, stored on ice and transferred to a –80 °C freezer. Just after the leaves had been collected, the plants were randomly assigned to one of three infestation treatments: *P. rapae*, *P. xylostella*, or *M. brassicae*. All caterpillars were obtained from the respective insect cultures of the Laboratory of Entomology, Wageningen University and were cultured on *B. oleracea* variety *gemmifera* cv. Cyrus. Each plant was infested with 10 neonate caterpillars of the herbivore species and with five replicates per cultivar–herbivore species combination. Distance between plants and the 1-cm layer of water in the trays prevented caterpillars from moving between plants. After 6 days (t = 1), a second newest fully grown leaf, on which caterpillars had been feeding, was collected following the same procedure as mentioned at the beginning of this section. The herbivores were collected, weighed individually to the nearest 0.1 mg, and placed back onto the plants from which they originated. On day 12 (t = 2), we collected a third fully grown leaf that was damaged by the caterpillars and was positioned as first leaf below the leaf collected on day 6. The caterpillars were surveyed daily for survival and development until pupation. When caterpillars had pupated, the date of pupation was noted and pupae were weighed to the nearest 0.1 mg. Caterpillars of *M. brassicae* pupate in the soil. Therefore, caterpillars of *M. brassicae* that reached the final instar were placed in plastic boxes containing a layer of 5 cm of peat soil and were provided ad libitum with excised leaf material of the plant they originated from until pupation.

The performance experiment was repeated starting on 6 April 2005 with a new set of 8-week-old plants. Ten first instars were placed on each of 10 plants for each cultivar (using 100 caterpillars per cultivar), that is, twice as many as in the first experiment, and surveyed for growth and development. As there were 900 caterpillars per species, on 1 day we could only perform weighings of a single caterpillar species. Therefore, caterpillars were weighed when the fastest growing caterpillar of that particular species reached the fourth instar. This resulted in weighing of *P. xylostella* on day 7, *P. rapae* on day 9, and *M. brassicae* on day 20 since the larvae had been introduced on the plants. For these caterpillars, we also measured the pupal weight and number of days to reach pupation since introduction on the plants.

Glucosinolate profiling

The collected leaves that originated from 135 plants, consisting of eight cultivars and *B. nigra* that were each sampled at three time intervals, were freeze dried and ground to a fine powder. Fifty mg of ground leaf material per sample was dissolved in methanol. The extract was desulfated on a DEAE-Sephadex A25 column (Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands) and the GS content was assessed by high performance liquid chromatography (HPLC), using the method described by van Dam et al. (2004). Five concentrations of sinigrin (sinigrin monohydrate; Sigma, St. Louis, MO, USA) were desulfated following the same protocol as the samples and were used as an external standard. Glucosinolate detection was performed with a Photodiode Array detector (200–350 nm) with 229 nm as the integration wavelength. We used the correction factors at 229 nm from Buchner (1987) and the EC (European Community, 1990) to calculate the concentrations of the GSs. Desulfoglucosinolate peaks were identified by comparison of HPLC retention times and ultraviolet spectra with standards kindly provided by M. Reichelt (Max Planck Institute for Chemical Ecology, Jena, Germany) and a certified rapeseed standard (code BCR-367R; Community Bureau of Reference, Brussels).

Statistical analysis

Performance parameters of each herbivore species were analysed using SPSS 12.0.1 (Chicago, IL, USA) by running separate tests for each parameter per herbivore species. Analysis of variance (ANOVA) with post-hoc Tukey tests for cultivar comparisons were applied to analyse caterpillar and pupal mass. Kruskal–Wallis tests were applied to analyse the time until caterpillars pupated on different cultivars. Data of the two replicates of the performance experiment were analysed separately, because caterpillars from the second series were growing slightly faster and were weighed at different time-points compared to the first series. Nevertheless, ranking of cultivars for performance of each caterpillar species was similar for both series. Because a larger number of caterpillars were weighed in the second series, we only present detailed herbivore data of this experiment.

The quantitative and qualitative GS content of the plants and the effect of each herbivore species on GS content were analysed using two models. In both models, the dependent variable, GS concentration, was normalized by an arcsin square root transformation. To analyse the constitutive differences in GS profile of the eight *B. oleracea* cultivars and *B. nigra*, we applied a multiple ANOVA (MANOVA) on the amount of GS without any herbivore damage (t = 0). To analyse the effect that different herbivore species had on the amount of GS in different *Brassica* species, a mixed model with repeated measurement structure subjected to plants (6 and 12 days) was used. The GS concentration was modelled by the factors cultivar, herbivore species, time, and their interactions by using the PROC MIXED function of SAS 9.1. Average total-, alkenyl-, and indolyl-glucosinolate concentrations of the cultivars were correlated with average caterpillar mass per cultivar for each herbivore species, using linear regression.

Results

Herbivore performance

We found that caterpillar performance of the three herbivore species depended on the *Brassica* cultivar on which they were feeding, which is reflected in the caterpillar mass (ANOVA, *P. rapae*: $F_{8,550} = 28.039$, $P<0.001$; *P. xylostella*: $F_{8,255} = 31.728$, $P<0.001$; and *M. brassicae*: $F_{8,278} = 9.502$, $P<0.001$). The development time until pupation of these caterpillars showed a reversed pattern; caterpillars that fed on a cultivar resulting in low caterpillar mass had a longer development time (Kruskal–Wallis test, *P. rapae*: $H = 113.644$, d.f. = 8, $P<0.001$; *P. xylostella*: $H = 135.759$, d.f. = 8, $P<0.001$; and *M. brassicae* $H = 68.217$, d.f. = 8, $P<0.001$) (Figure 1). Although the ranking order of performance over the *Brassica* cultivars and species tested was relatively similar for all three herbivores, post-hoc Tukey tests for cultivar effects on fourth-instar mass also revealed differences between the herbivores. The two *Brassica* specialists (*P. xylostella* and *P. rapae*) performed well on *B. nigra*, which had the highest concentration of GS, whereas the generalist *M. brassicae* had a low performance on *B. nigra*. Furthermore, the cultivar Jersey Queen supported lower performance of *M. brassicae* and *P. xylostella* than of *P. rapae*. Over the eight *B. oleracea* cultivars tested, the specialist *P. xylostella* and generalist *M. brassicae* were similar in the ranking order of performance and differed from the specialist *P. rapae* (Figure 1).

Pupal masses of all three herbivore species correlated negatively with the number of days before pupation occurred (Pearson correlation coefficient, *P. rapae*: $r = -0.252$, $P<0.001$; *M. brassicae*: $r = -0.194$, $P = 0.007$; and *P. xylostella*: $r = -0.373$, $P<0.001$). The cultivar on which the caterpillars were feeding affected pupal mass significantly in all three herbivores (ANOVA, *P. rapae*: $F_{8,352} = 5.682$, $P<0.001$; *P. xylostella*: $F_{8,187} = 9.392$, $P<0.001$; and *M. brassicae*: $F_{8,182} = 18.187$, $P<0.001$). Post-hoc Tukey tests revealed that feeding on cultivar Domia resulted in significantly lower pupal masses for *M. brassicae* whereas for *P. rapae* and *P. xylostella*, pupal mass on this cultivar was high or intermediate, respectively. On cultivar Stonehead, *M. brassicae* and *P. rapae* had low pupal masses, but *P. xylostella* reached a relatively high pupal mass (Figure 1).

Plant glucosinolate levels before and after herbivory

The eight *B. oleracea* cultivars and *B. nigra* differed in their constitutive composition of foliar GSs. Levels of GSs of 8-week-old plants ($t = 0$) were both qualitatively and quantitatively different between cultivars [MANOVA Pillai's trace: $F_{8,124} = 8.03$, $P<0.001$; glucoiberin (IBE): $F_{8,124} = 9.98$; sinigrin (SIN): $F_{8,124} = 93.00$; progoitrin (PRO): $F_{8,124} = 9.05$; glucoraphanin (RAPH): $F_{8,124} = 6.16$; gluconapin (GNA): $F_{8,124} = 10.88$; 4-hydroxyglucobrassicin (4OHGBC): $F_{8,124} = 13.14$; glucobrassicin (GBC): $F_{8,124} = 20.97$; 4-methoxyglucobrassicin (MGBC): $F_{8,124} = 9.36$; neo-glucobrassicin (NEOGBC): $F_{8,124} = 15.80$; and gluconasturtiin (NAS): $F_{8,124} = 24.83$, all $P<0.001$], with *B. nigra* having a 2–3-fold higher amount of total GS than the *B. oleracea* cultivars (Table 1). After 6 and 12 days of herbivore feeding, the GS levels of the cultivars were still significantly different for each compound (Table 2) and the concentration of all compounds had increased 2–5-fold. Furthermore, feeding by the three different caterpillar species resulted in different levels of all indole GS compounds measured (Table 2, Figure 2). For two of the indole compounds (4OHGBC and GBC), there was an interaction between caterpillar species and the cultivar on which they had been feeding. For most of the cultivars, feeding by *P. rapae* and *M. brassicae* resulted in higher amounts of indole GS, except for cultivar Christmas Drumhead. The amount of four compounds (PRO, GNA, 4OHGBC, and MGBC) increased only marginally between 6 and 12 days of herbivore feeding (Table 2). For alkenyl GS compounds, no differences were found between caterpillar species with the exception of an interaction between cultivar and caterpillar for sinigrin (Table 2). None of the performance parameters correlated with GS concentration of the *Brassica*-species and all three herbivores performed poorest on cultivar Rivera, which has low concentrations of GSs when grown under greenhouse conditions (Table 3).

Discussion

Our study revealed that the difference between caterpillar performance on native and cultivated plants as well as the ranking order of performance for *B. oleracea* cultivars varied for the three herbivore species. An increase in the number of days until pupation generally coincided with lower pupal mass. Performance differences were already reflected in caterpillar mass differences when the caterpillars of a particular species reached the fourth instar. However, in contrast to our hypothesis that plant defence affects generalists and specialists differently, the generalist *M. brassicae* and the specialist *P. xylostella* were similar in the ranking order of performance over the set of cultivated *B. oleracea* tested.

Whereas specialist herbivores are adapted to specific chemicals of their host plant, performance of generalists is typically affected by high levels of these chemical defences (van der Meijden, 1996; Schoonhoven et al., 2005). The 3-fold higher amount of GS in the native *B. nigra* indeed resulted in poor performance of the generalist *M. brassicae*

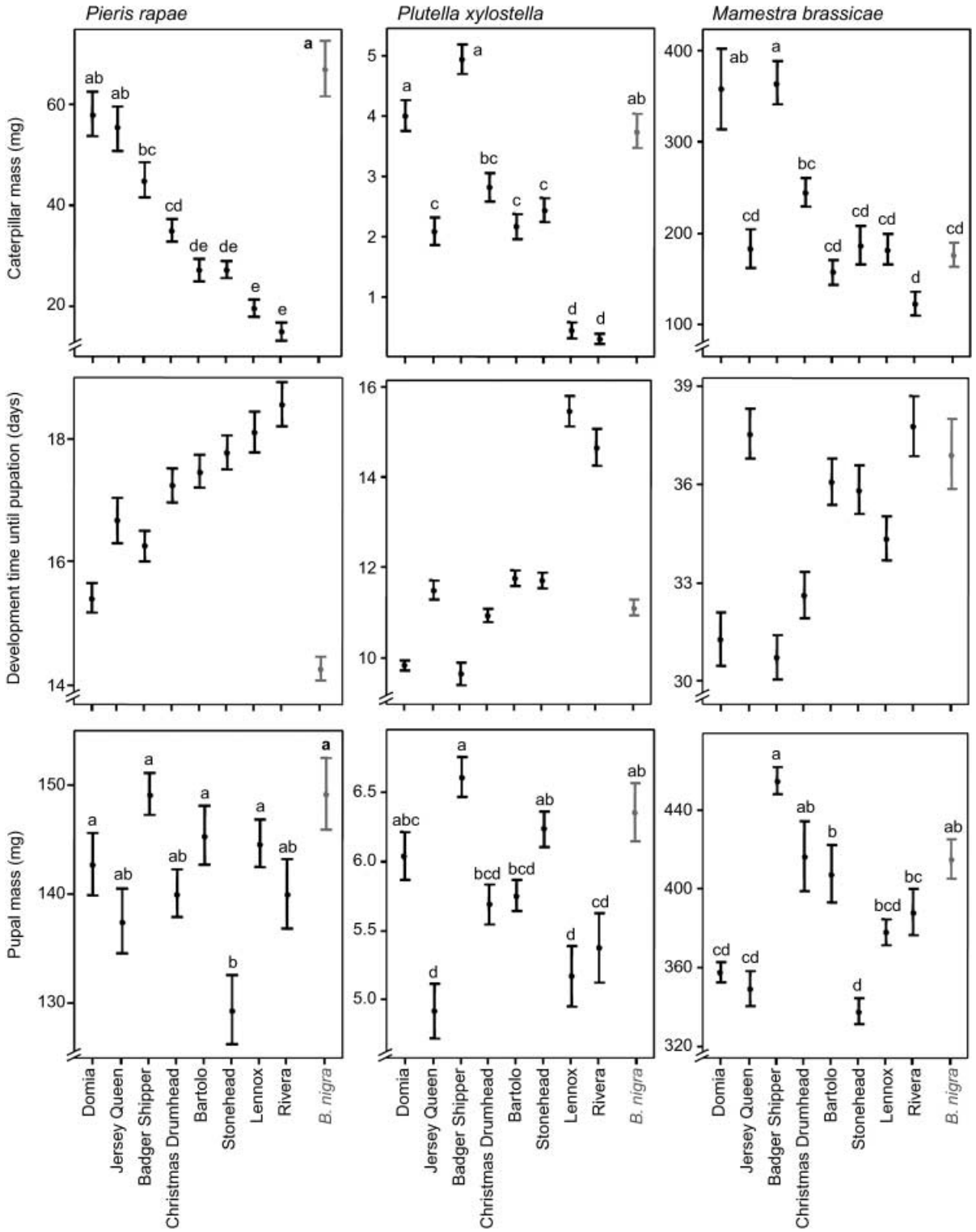

Figure 1 Performance of three herbivore species (*Pieris rapae*, *Plutella xylostella*, and *Mamestra brassicae*) on eight cultivars of *Brassica oleracea* (black) compared with the wild species *Brassica nigra* (grey). Per herbivore species from top to bottom, the panels present mean body mass (± SEM; n = 9–76) of caterpillars, time until pupation, and pupal mass. The cultivar sequence was arbitrarily based on caterpillar mass of *Pieris rapae*.

Table 1 Foliar glucosinolate profiles of 8-week-old plants of eight white cabbage cultivars of *Brassica oleracea* and the wild species *Brassica nigra*. Concentrations (μmol g^{-1} dry weight) of individual glucosinolates (GS) (SE in parentheses) are averages of 15 plants and differ significantly between cultivars [multiple analysis of variance (MANOVA) Pillai's trace: $F_{8,124} = 8.026$, $P<0.001$ for all compounds]. Scientific compound names are given for side chains only and all are complemented with '-glucosinolate' in their full scientific name

		Brassica oleracea variety *alba*								
Compound	Common name	Domia	Jersey Queen	Badger Shipper	Christmas Drumhead	Bartolo	Stonehead	Lennox	Rivera	*Brassica nigra*
Alkenyl glucosinolates										
3-Methylsulfinylpropyl	Glucoiberin (IBE)	1.41 (0.40)	1.45 (0.62)	1.67 (0.47)	0.12 (0.07)	1.20 (0.48)	0.01 (0.01)	0.83 (0.26)	0.03 (0.02)	–
2-Propenyl	Sinigrin (SIN)	1.57 (0.33)	1.33 (0.36)	0.58 (0.15)	0.33 (0.09)	0.68 (0.14)	3.72 (0.57)	0.42 (0.06)	0.15 (0.05)	28.33 (3.42)
R-2-Hydroxy-3-butenyl	Progoitrin (PRO)	0.01 (0.01)	0.56 (0.21)	0.46 (0.19)	–	0.03 (0.02)	0.01 (0.01)	–	0.04 (0.02)	–
4-Methylsulfinylbutyl	Glucoraphanin (RAPH)	0.04 (0.03)	3.17 (1.58)	1.10 (0.35)	0.01 (0.00)	0.14 (0.08)	–	0.08 (0.04)	0.01 (0.01)	–
3-Butenyl	Gluconapin (GNA)	0.13 (0.03)	0.82 (0.25)	0.19 (0.07)	0.06 (0.03)	0.05 (0.02)	0.07 (0.02)	–	0.06 (0.02)	–
Indolyl glucosinolates										
4-Hydroxy-3-indolylmethyl	4-hydroxyglucobrassicin (4OHGBC)	0.05 (0.01)	0.23 (0.05)	0.13 (0.02)	0.04 (0.01)	0.09 (0.02)	0.01 (0.00)	0.11 (0.02)	0.09 (0.03)	0.26 (0.05)
3-Indolylmethyl	Glucobrassicin (GBC)	0.09 (0.02)	1.22 (0.30)	0.24 (0.04)	0.08 (0.02)	0.20 (0.04)	0.03 (0.01)	0.11 (0.01)	0.16 (0.03)	0.13 (0.05)
1-Methoxy-3-indolylmethyl	Neo-glucobrassicin (NEOGBC)	0.01 (0.00)	0.03 (0.01)	0.33 (0.14)	0.02 (0.01)	0.01 (0.01)	–	0.02 (0.01)	0.01 (0.00)	–
4-Methoxy-3-indolylmethyl	4-methoxyglucobrassin (MGBC)	0.08 (0.01)	0.10 (0.03)	0.09 (0.01)	0.02 (0.01)	0.02 (0.00)	0.02 (0.00)	0.02 (0.01)	0.10 (0.04)	0.09 (0.03)
Aromatic glucosinolates										
2-Phenylethyl	Gluconasturtiin (NAS)	–	–	–	–	–	–	–	–	0.14 (0.04)
Total GS		3.38 (0.74)	8.91 (2.74)	4.79 (0.88)	0.67 (0.19)	2.42 (0.66)	3.85 (0.61)	1.58 (0.34)	0.64 (0.16)	28.95 (3.47)

Table 2 F-test for repeated measurement Mixed model analysis of the concentration of glucosinolates (GS) in the plants, testing the factors cultivar, caterpillar species feeding on the plant and the time of measurement after herbivore feeding, as well as the factorial interactions

	Factor						Interaction							
	Cultivar (1) (d.f. = 8)		Caterpillar (2) (d.f. = 8)		Time (3) (d.f. = 1)		(1*2) (d.f. = 16)		(1*3) (d.f. = 8)		(2*3) (d.f. = 2)		(1*2*3) (d.f. = 16)	
Compound	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Alkenyl glucosinolates														
Glucoiberin (IBE)	21.68	<0.001[1]	1.24	0.29	0.12	0.74	1.22	0.25	0.34	0.95	0.18	0.83	0.63	0.86
Sinigrin (SIN)	59.43	<0.001[1]	1.90	0.15	0.27	0.60	2.72	<0.001[1]	0.84	0.57	1.76	0.18	1.35	0.17
Progoitrin (PRO)	5.15	<0.001[1]	0.54	0.58	10.17	0.002[1]	0.97	0.49	1.40	0.20	1.46	0.23	0.68	0.81
Glucoraphanin (RAPH)	16.03	<0.001[1]	1.77	0.17	2.64	0.11	0.87	0.61	0.24	0.98	0.32	0.73	0.26	0.99
Gluconapin (GNA)	13.41	<0.001[1]	0.79	0.46	4.35	0.04[1]	1.28	0.28	0.23	0.99	0.44	0.64	0.35	0.99
Indolyl glucosinolates														
4-Hydroxyglucobrassicin (4OHGBC)	9.28	<0.001[1]	13.29	<0.001[1]	140.10	<0.001[1]	2.11	0.009[1]	4.14	<0.001[1]	4.01	0.02[1]	1.08	0.38
Glucobrassicin (GBC)	13.41	<0.001[1]	7.92	<0.001[1]	1.03	0.31	2.29	0.004[1]	1.05	0.40	1.26	0.29	0.69	0.81
4-Methoxyglucobrassicin (MGBC)	8.22	<0.001[1]	9.98	<0.001[1]	33.54	<0.001[1]	1.03	0.43	0.44	0.90	3.54	0.03[1]	0.14	0.99
Neo-glucobrassicin (NEOGBC)	15.74	<0.001[1]	11.34	<0.001[1]	1.52	0.22	1.05	0.41	1.12	0.35	0.13	0.88	0.38	0.99

[1]P-values are significant at $\alpha = 0.05$.

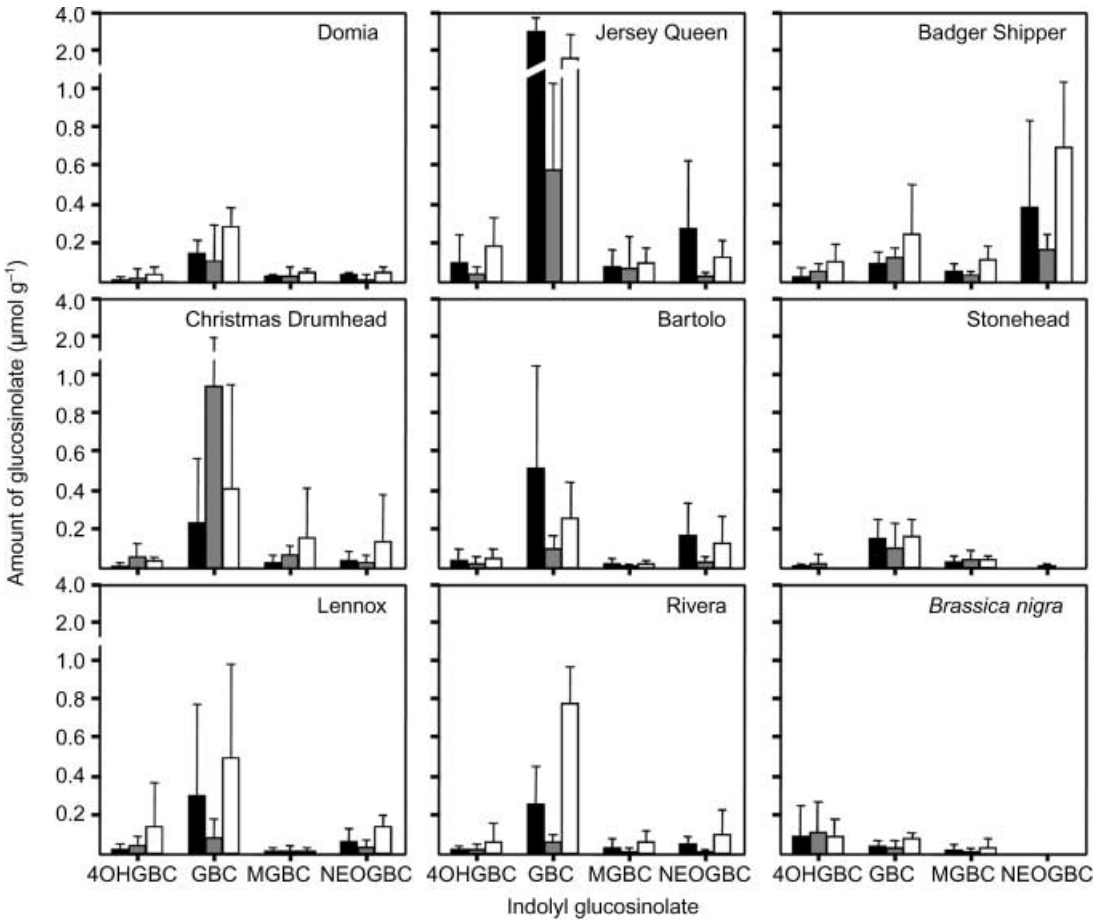

Figure 2 Indole glucosinolate content of eight *Brassica oleracea* cultivars and *Brassica nigra* after 6 days of feeding by different herbivores: *Pieris rapae* [(black bars (average + SEM)], *Plutella xylostella* (grey bars), and *Mamestra brassicae* (white bars). *Pieris rapae* and *M. brassicae* feeding resulted in significantly higher amounts of each indole glucosinolate compound than when *P. xylostella* was feeding on the plants (Mixed model, $P<0.001$).

and matched earlier findings of a negative correlation between performance and foliar concentration of GSs for generalists (Chew, 1988; Giamoustaris & Mithen, 1995; Li et al., 2000). The better performance of *P. rapae* and intermediate performance of *P. xylostella* on *B. nigra* matched the general pattern of tolerance of specialists to GSs (Chew, 1988; Bodnaryk, 1997; Li et al., 2000; Kliebenstein et al., 2002; Müller & Sieling, 2006; Gols et al., 2008) or the stimulating effect on caterpillar feeding by these compounds (van Loon et al., 2002). However, within cultivated plants the performance of the three herbivore species did not correlate with the concentrations of total GS or any single GS compound, nor was the performance of *M. brassicae* the poorest on *B. nigra*.

Differences in herbivore performance could also not be correlated with GS concentrations measured after herbivory. After feeding by the three herbivores, indole GS concentrations were different for the herbivore species. *Mamestra brassicae* and *P. xylostella*, which exhibited a similar ranking order of performance on the eight cultivars, were different in their effect on indole GSs. Depending on the type of herbivore feeding on the plant, indole GS concentrations had increased 2–4-fold after 6 days of herbivory. Indole GS concentrations in leaves of *Brassica* are known to be stable over crop development time (Fieldsend & Milford, 1994), suggesting that the extent of the increases we found are due to induced response of the plant after herbivory in all three herbivore treatments rather than associated with plant age. The induction of indole GS has been related to biotic stress such as herbivory (Koritsas et al., 1991; Rostás et al., 2002; Traw & Dawson, 2002; Mewis et al., 2006; Kim & Jander, 2007) or plant hormones that regulate biotic stress responses (Bodnaryk, 1994; Loivamäki et al., 2004). As hypothesized, feeding by

Table 3 Regression analysis of caterpillar mass of the three herbivores correlated to (A) the constitutive and (B) the induced concentration of foliar glucosinolates

	Total glucosinolates			Alkenyl glucosinolates			Indolyl glucosinolates		
Herbivore species	R	F	P	R	F	P	R	F	P
(A) Constitutive glucosinolate content									
Pieris rapae	0.62	4.33	0.08	0.60	4.01	0.09	0.42	1.50	0.26
Plutella xylostella	0.37	1.11	0.33	0.37	1.10	0.33	0.11	0.08	0.78
Mamestra brassicae	–0.13	0.12	0.74	–0.13	0.12	0.74	0.02	0.00	0.96
(B) Induced glucosinolate content									
P. rapae	0.53	2.70	0.14	0.46	1.83	0.22	0.25	0.48	0.51
P. xylostella	0.23	0.39	0.55	0.23	0.37	0.56	0.01	0.00	0.98
M. brassicae	–0.17	0.20	0.67	–0.21	0.32	0.59	0.32	0.79	0.40

the generalist *M. brassicae* resulted in higher concentrations of indole GS than feeding by the specialist *P. xylostella*. However, the other specialist (*P. rapae*) elicited a similar response as *M. brassicae*, making the degree of host plant specialization not a plausible explanation for differential response of defence expression in plants after herbivory. The suggested differential induction of indole GS may have resulted from herbivore differences in the amount and pattern of damage they inflicted, as well as differences in constituents of oral secretions (Mattiacci et al., 1995). The first larval stages of *P. xylostella* are mining; due to their much smaller size than *M. brassicae* or *P. rapae* caterpillars, the later stages of *P. xylostella* cause less damage, distributed over many small holes (Olsson & Jonasson, 1994). The observed similarity in effect on indole GS for the two categories of host-plant specialization may be caused by similarity in feeding behaviour of *M. brassicae* and *P. rapae* caterpillars.

Although herbivores differentially affected the concentration of indole GS, the effectiveness of indole GS as chemical defence compound against caterpillars may be weak; interaction of myrosinase with indole GS results in only small amounts of toxic isothiocyanates that are unstable in biological fluids (Bones & Rossiter, 2006; N Agerbirk, pers. comm.). However, recent studies revealed that intact indole GS compounds deter aphids and negatively affect aphid reproduction even in the absence of myrosinase (Kim & Jander, 2007). Breakdown of unstable indole GS in the absence of myrosinase results in yet unknown compounds (Kim & Jander, 2007), which may also be effective against other herbivores. Other defensive chemicals, differences in primary metabolites serving as nutrients, and synergistic effects between compounds may explain similarity in performance across host-plant specialization as found in this study. Herbivores, both specialists and generalists, are affected by enhanced nutrient levels in cultivars that resulted from artificial selection (Benrey et al., 1998; Fahey et al., 2001; Schoonhoven et al., 2005). Furthermore, cultivated *Brassica* plants were found to have higher levels of proteinase inhibitors than their native congeners (Broadway, 1989) and these compounds were found to affect both generalist and specialist herbivores negatively (Broadway & Colvin, 1992). Microarray studies revealed that the expression of genes coding for proteinase inhibitors in response to *P. rapae* feeding was higher in cultivar Rivera than in Christmas Drumhead (Broekgaarden et al., 2007) and may account for poor performance of all three herbivores on cultivar Rivera. Other physical traits such as thickness of the epicuticular wax layer may have reduced the performance of some species (Picoaga et al., 2003). The difference in performance between the two specialists may further be explained by a difference in resistance mechanism against toxins in *P. rapae* and *P. xylostella* (Agrawal, 2000; van Dam et al., 2000; Agrawal & Kurashige, 2003). *Plutella xylostella* is known to desulfate GS and may thereby prevent that the breakdown of GS by myrosinase results in highly toxic compounds (Ratzka et al., 2002), whereas *P. rapae* redirects the myrosinase-catalysed GS hydrolysis to form nitriles instead of the more toxic isothiocyanates (Wittstock et al., 2004). Both detoxification strategies may result in different amounts of breakdown products of GS that can negatively affect performance of specialists (Li et al., 2000; Agrawal & Kurashige, 2003). Despite the lack of a clear correlation between performance and amount of a single type of secondary chemicals, that is, GS, we have shown that the set of defence traits of plants may affect performance of two herbivores similarly across different degrees of host-plant specialization and that this similarity may also occur for plant responses after herbivory.

Although the difference in indole GS expression found in this study did not correlate with larval performance of

herbivores, these compounds may still function as enhanced defence when reducing oviposition acceptance of plants by herbivores. Glucosinolates have been found to reduce the acceptance of brassicaceous plants by generalist butterflies (Mithen et al., 1995), but on the other hand act as oviposition cues for specialist butterflies (van Loon et al., 1992; Giamoustaris & Mithen, 1995; Moyes et al., 2000; Renwick et al., 2006; Bruinsma et al., 2007). Future studies on plant responses to herbivory in terms of GS content should therefore address the role of indole GS as directed against subsequent attackers or natural enemies of attackers to further elucidate the effects of inducible indole GS.

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