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INFLUENCE OF FOLIAR GLUCOSINOLATES IN OILSEED RAPE AND MUSTARD ON FEEDING AND GROWTH OF THE BERTHA ARMYWORM, *Mamestra configurata* WALKER

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Abstract—The relationship between host plant glucosinolate profile and feeding and growth of the Bertha armyworm, *Mamestra configurata* Walker was investigated using eight cultivated rape and mustard varieties. Mean larval weights of neonates reared on intact rosette-stage plants were significantly different on the different species in the order *Brassica juncea* < *Sinapis alba* < *B. napus* < *B. campestris*. While *B. juncea* was least preferred, *S. alba* was significantly more attractive to neonate larvae in choice tests. Relative consumption and growth rates of fourth-instar larvae were also reduced on *B. juncea* foliage. Other differences were dependent on the plant growth stage. Neonate preference was not correlated to total glucosinolate levels, but rather to the concentrations of isothiocyanate-releasing glucosinolates. However, the relationship between consumption and glucosinolate levels was inconsistent. Relative growth rate was negatively correlated to total glucosinolate content for stage 3 and 4 foliage—mainly due to the concentration of isothiocyanate-releasing glucosinolates. The relative importance of isothiocyanate-releasing glucosinolates was verified by rearing neonates on meridic diets containing equimolar concentrations of sinigrin, its metabolite, allyl isothiocyanate, and indole-3-carbinol, metabolite of 3-indolylmethyl glucosinolate. Sinigrin and allyl isothiocyanate in the diet produced virtually identical negative weight vs. concentration regression lines. No such dose–response effect was observed with indole-3-carbinol. The data suggest that foliar isothiocyanate-releasing glucosinolates may provide some degree of plant protection from polyphagous insects.

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Key Words—*Brassica campestris*, *B. juncea*, *B. napus*, Bertha armyworm, canola, glucosinolate, insect-plant interactions, isothiocyanate, *Mamestra configurata*, Lepidoptera, Noctuidae, mustard, *Sinapis alba*, thiocyanate.

INTRODUCTION

Glucosinolates and their volatile degradation products have historically been implicated as kairomones for insects that specialize on cruciferous plants (Verschaffelt, 1911; Nayar and Thorsteinson, 1963; Traynier, 1965, David and Gardiner, 1966; Read et al., 1970; Feeny et al., 1970; Nault and Styler, 1972; Rygg and Somme, 1972; Hicks, 1974; Nair and McEwen, 1976; Finch, 1978; Reed et al., 1989; Traynier and Truscott, 1991). While the presence of these compounds undoubtedly contributes to host plant recognition, recent laboratory studies have revealed that the host selection responses of some crucifer specialists are not necessarily side-chain- or dose-dependent (Reed et al., 1989; Bodnaryk and Palaniswamy, 1990). Field experiments comparing high- and low-glucosinolate rape cultivars have shown that infestation levels of certain crucifer pests are not related to total glucosinolates (Ahman, 1982; Lamb, 1988; Williams, 1989; Butts and Lamb, 1990). Moreover, the widespread cultivation of low-glucosinolate rape varieties (canola) on the Canadian prairies has not led to differences in crucifer pest infestation levels (Lamb, 1989).

Allelochemicals in plants may also affect the palatability of the substrate as well as the growth of the herbivore, two important parameters of insect resistance. In this mode, glucosinolates have been shown to function as allomones for some nonspecialist insects (Nault and Styler, 1972; Blau et al., 1978). Hypothetically, the presence of glucosinolates in the foliage of the crop may be advantageous if the consumption and growth of foliage-feeding insects can be inhibited. In the present study we have examined foliar glucosinolates qualitatively and quantitatively, and investigated their relationship to food consumption and growth of a Canadian canola pest, the Bertha armyworm, *Mamestra configurata* Walker. *M. configurata* is a nonspecialist insect that includes brassicaceous plants in its host range.

Eight cultivars representing four species of cultivated oilseed rape and mustard were chosen for our study, the main criteria for their selection being their differences in glucosinolate profile. Essentially two types of glucosinolates, based on their metabolic products following the action of endogenous myrosinase (EC 3.2.3.1), dominate in the foliage of the plants studied. Unsubstituted alkenyl and aryl glucosinolates produce isothiocyanates that are generally insecticidal (Lichtenstein et al., 1962, 1964; Nayar and Throsteinson, 1963; Lowe et al., 1971; Ahman, 1986; Bartelt and Mikolajczak, 1989), while those possessing an indolyl or hydroxy-aryl side chain ultimately degrade to form

R-alcohols and thiocyanate ion (at the pH range of the lepidopteran alimentary tract), whose biological activity is less well known. This study examines the relationship of biological activity in *M. configurata* to foliar concentrations of total glucosinolates, isothiocyanate-releasing glucosinolates, and thiocyanate-releasing glucosinolates. In addition, we attempt to verify the plant foliage effects with feeding studies using pure compounds augmented to meridic diet. Allyl isothiocyanate, the metabolite of allyl glucosinolate (sinigrin), and indole-3-carbinol, the metabolite of 3-indolylmethyl glucosinolate, were commercially available examples of the two types of glucosinolate metabolites of interest. Sinigrin, also commercially available, was used as an example of an intact glucosinolate.

METHODS AND MATERIALS

Insect Culture. A laboratory culture of *Mamestra configurata* was maintained at 20°C with a 16:8 (hr light–dark) photoperiod on an agar-based meridic diet (Velvetbean caterpillar diet, BioServ No. F9795, BioServ, Frenchtown, N.J.) augmented with 1.5% Vanderzant vitamin mixture and 1% alfalfa meal. Lamb's quarters (*Chenopodium album* L.) were presented to moths for oviposition (Bucher and Bracken, 1976). Eggs were removed from the leaves prior to hatching so that insects used for experiments had no previous experience with plant material.

Insect Growth on Greenhouse-Grown Plants. Seeds of *Brassica napus* cv. Westar, Regent, and Midas, *B. campestris* cv. Candle and Tobin, *B. juncea* cv. Lethbridge 22A, and commercial brown mustard and *Sinapis alba* cv. Gisilba were sown in vermiculite. Cotyledon stage plants were individually transplanted into 10-cm pots of a homogenous, sterilized soil mix and grown to the four-leaf rosette stage under high-intensity sodium vapor lamps (40,000 lux at plant level). Soluble 20–20–20 (N:P:K) fertilizer was applied weekly.

Five neonate *M. configurata* larvae were placed on each of 10 plants per variety. A screen-bottomed, clear plastic beverage cup was inverted over each plant and embedded into the soil to confine the insects. The plants and insects were transferred to a controlled environment growth chamber with a 16:8 (hr light–dark) photoperiod (16,000 lux) and a constant temperature of 20°C for seven days, after which time the insects were weighed. The data were subjected to ANOVA with individual degree of freedom (idf) tests (orthogonal contrasts) for differences based on species and cultivar within species.

Biological Assays with Field-Grown Foliage. Seeds of the previously described varieties were sown in the field at the Plant Science Field Laboratory, University of British Columbia campus, Vancouver. Blocks that included plots of each cultivar were sequentially planted to ensure that foliage of the desired

plant growth stages were available for testing when insect stocks were available. The preemergent herbicide trifluralin (Treflan E.C.) was applied to control weeds. Soluble 20–20–20 (N : P : K) fertilizer was applied weekly. Plots were subdivided such that individual plants were sampled once only.

Three broad plant growth stages (Harper, 1973) were examined in the study: rosette (stage 2), stem elongation (stage 3), and flowering (stage 4). Two blocks (= replicates in time) were sampled for each growth stage, except the flowering stage due to limitations in the insect culture. For each sampling interval, the following biological assays were carried out within a period of three days:

1. Neonate choice test. Leaf disks were punched from foliage using a 1.6-cm cork borer. Disks of each cultivar (eight in total) were arranged randomly and evenly spaced around the perimeter of a Petri dish (14 cm) on a moist filter paper disk. (12.5 cm, Whatman No. 1). One hundred neonate *M. configurata* larvae were introduced into the center of each dish, which was covered and left in darkness at 20°C for 16 hr, after which time the numbers of larvae on each leaf disk were recorded. Ten replicate Petri dishes (1000 insects) were set up for each sampling interval. Because the treatment variances were proportional to the means, the data were square-root transformed $(x + 0.5)^{0.5}$ prior to analysis.

2. Fourth-Instar Nutritional Indices. Individual leaves were excised from the plants and placed in a 10-cm Petri dish on a moist filter paper disk. A fourth-instar *M. configurata* larva, within a weight range of 13–22 mg, was placed on the leaf and allowed to feed for two days at 20°C with a 16 : 8 (hr light–dark) photoperiod. The following nutritional indices (Waldbauer, 1968) were calculated on a dry weight basis using the insect's weight at the start of the feeding period as the reference weight (Farrar et al., 1989):

$$\text{Relative consumption rate (RCRi)} = \frac{\text{food ingested/insect initial weight}}{\text{no. of days}}$$

$$\text{Relative growth rate (RGRi)} = \frac{\text{weight gained/initial weight}}{\text{no. of days}}$$

Sixteen insects per treatment were tested at each sampling date (replicate).

For the two described assays, ANOVA with idf tests was performed for each plant growth stage, and the treatment (cultivar) means were regressed against glucosinolate concentration for each block.

Glucosinolate Analysis. Bulk samples of foliage (a minimum of 20 plants) from each sampling interval were collected for analysis of glucosinolates. Foliage was quick-frozen with liquid N_2, lyophilized, and ground to a homogenous powder in a blender (maximum particle size 1 mm). Freeze-dried material was stored at −20°C prior to analysis.

Samples (100 mg) of powdered plant material were heated for 2 min in a boiling water bath before being extracted thrice with 3 ml of boiling 70% methanol for 2 min per extraction at the solvent boiling point. Methanol was removed from the pooled extracts by heating in a 40°C water bath under a stream of N_2. The aqueous solution was made up to 6 ml with water, and 125 μl of 0.6 M barium-lead acetate solution [0.3 M $Ba(CH_3COO)_2$ and 0.3 M $Pb(CH_3COO)_2$] was added to precipitate phenolics and free sulfate (McGregor, 1985). Following centrifugation, the supernatant was used for glucosinolate determination. Extraction efficiency averaged 83% as determined by HPLC of recovered desulfosinigrin from sinigrin-spiked Westar canola foliar slurries. Reported quantifications are the means of two separate extractions and analyses.

Total Glucosinolates. Total glucosinolates were quantified by the thymol method (Brzezinski and Mendelewski, 1984; Tholen et al., 1989). Glucosinolates were further purified from the supernatant by ion exchange on DEAE-Sephadex A-25 minicolumns regenerated with water. After washing with 2 × 1 ml of 30% w/v formic acid and 4 × 1 ml water, glucosinolates were eluted with 5 × 1 ml 0.3 M potassium sulfate. Aliquots (0.5 ml) were mixed with 100 μl of ethanolic 6% thymol and 2 ml 78% v/v sulfuric acid, and incubated for 35 min in a 93°C water bath. The absorbance of the solution was measured at 505 nm, and the linear regression equation of a standard curve of sinigrin was used for quantification.

Individual Glucosinolates. These were determined by HPLC of their desulfo derivatives combining the methods of McGregor (1985) and Bjerg and Sorensen (1987). The supernatant was applied to a 100-mg (dry weight) minicolumn of DEAE-Sephadex A-25 regenerated in 0.02 M sodium acetate buffer (pH 5.0). After washing with 4 × 1 ml of water and 4 × 1 ml of the sodium acetate buffer, a 0.5-ml aliquot of aryl sulfatase (EC 3.1.6.1) (Sigma, H-1) solution, prepared as per McGregor (1985) was applied to the column. After 20 hr in darkness, desulfoglucosinolates were eluted with 4 × 1 ml water, concentrated to dryness using a freeze-drier, and taken up in 400 μl water in an ultrasonic bath. Twenty-microliter aliquots were analyzed by HPLC.

The analytical column used was either a Waters Nova-Pak or a Phenomenex Bondclone (both C-18, 3.9 × 150 mm). This was preceded by a 2 × 20-mm guard column packed with C-18 pellicular packing (Perisorb, Upchurch Scientific). The solvent program consisted of water and acetonitrile at the following ratios: 99:1 (water–acetonitrile) for 8 min, followed by a linear gradient to 76:24 at 34 min, and held at this ratio until 36 min. The flow rate was 0.6 ml/min, and the absorbance of desulfoglucosinolates was monitored at 226 nm. Quantifications were based on response factors relative to an internal standard of *O*-nitrophenyl-β-D-galactopyranoside (Sigma) as described by McGregor (1985). Identification of major peaks (Figures 1 and 2 below) was confirmed by HPLC-MS as per the methods of Hogge et al. (1988a, b). The quantities of

isothiocyanate- or thiocyanate-producing gsls from individual peaks were added and these values used in the correlation analyses with the biological data.

Bioassay of Pure Compounds in Meridic Diet. Sinigrin monohydrate (Sigma), allyl isothiocyanate (Eastman), and indole-3-carbinol (Sigma) were tested against *M. configurata* in meridic diets at concentrations of 0.5, 1.0, and 1.5 μmol/g wet weight. Sinigrin and indole-3-carbinol were dissolved in methanol and added to the dry mix of the previously described standard diet and the methanol allowed to evaporate for 5 hr prior to mixing with the agar–water portion. A control diet was prepared by adding methanol only. Allyl isothiocyanate, which is extremely volatile, was added directly to the molten diet prior to gelling. The solidified diets were divided into 30-ml plastic cups and a single neonate larva was introduced into each cup. The tightly capped cups were placed in a humidified plastic box inside a controlled environment chamber at 23°C with a 16:8 hr light–dark photoperiod for eight days, after which time the insects were weighed. Forty insects were used for each treatment, and the mean larval weights for each treatment were regressed against concentration for each compound.

RESULTS

HPLC glucosinolate profiles of our plants are qualitatively similar to those published for *B. juncea* and *B. napus* cv. Midas (Sang et al., 1984). The dominant glucosinolate in *B. juncea* is allyl glucosinolate (sinigrin, **1**); in *S. alba*, OH-benzyl glucosinolate (sinalbin, **2**) dominates (Figure 1). Among the rape species, the indolyl glucosinolates predominate, particularly 3-indolylmethyl glucosinolate **(8)** and its 4-hydroxy analog **(4)** (Figure 2).

The growth rate of neonate *M. configurata* as measured by larval weight after seven days was influenced by the cultivar upon which the larvae fed (Figure 3). Orthogonal contrasts revealed significant differences between mean larval weights by plant species in the order: *B. juncea* < *S. alba* < *B. napus* < *B. campestris*. There were no significant differences between cultivars within *B. juncea* or *B. napus*. However, within *B. campestris*, larvae reared on Tobin were significantly larger than those reared on Candle.

The relative acceptability of the various host plants to neonate larvae was indicated by the choice tests with leaf disks (Figure 4). Larval distribution on host plants of all growth stages tested was similar to the ranking of larval weights in the growth experiment (Table 1). The number of larvae found on the *B. juncea* leaf disks was significantly lower than on the other species. However, *S. alba* consistently attracted significantly more insects than the other species. In agreement with the results of the growth experiment, *B. campestris* was more acceptable than *B. napus* rosette stage foliage. This ranking was reversed for

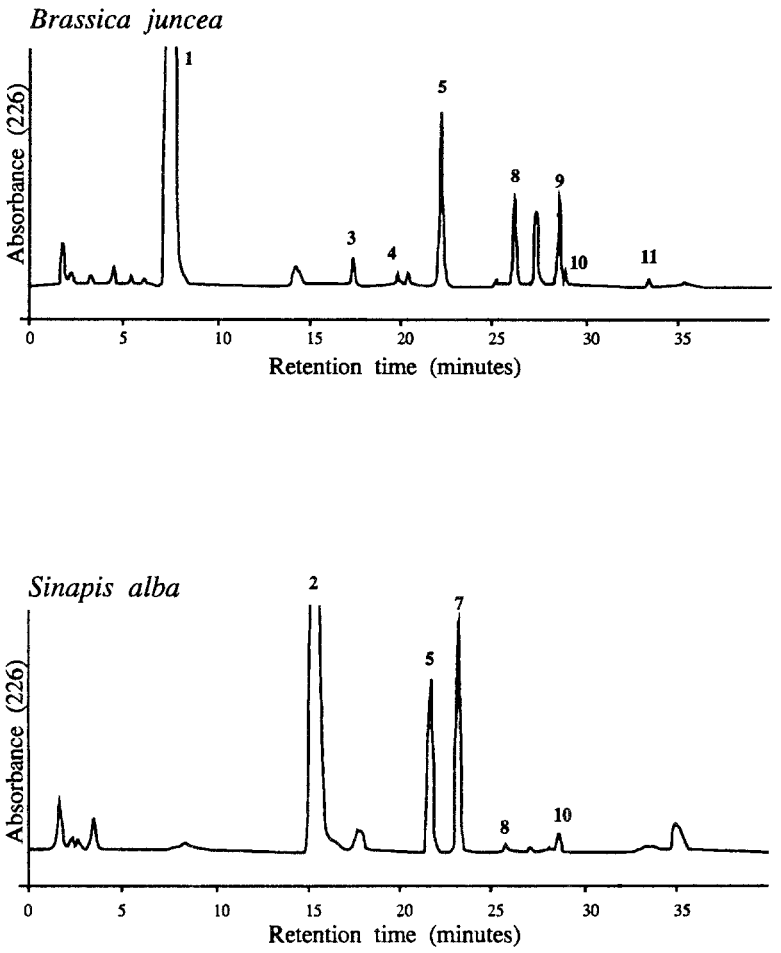


FIG. 1. Representative HLPC chromatograms of glucosinolate profiles for host plant species and/or cultivars used in the study. Peak numbers represent the following desulfoglucosinolates: **1,** allyl (sinigrin); **2,** OH-benzyl (sinalbin); **3,** 3-butenyl; **4,** 4-OH-3-indolylmethyl; **5,** ONPGal (internal standard); **6,** 4-pentenyl; **7,** benzyl; **8,** 3-indolylmethyl; **9,** 2-phenylethyl; **10,** 4-methoxy-3-indolylmethyl; **11,** 1-methoxy-3-indolylmethyl.

stage three foliage, and there was no significant difference between the attractiveness of the two species for flowering stage foliage. Within *B. napus*, the numbers of larvae on the high-glucosinolate cultivar Midas did not differ significantly from the low-glucosinolate cultivars, Regent and Westar. However, with stage 3 foliage, Westar attracted significantly more larvae than Regent.

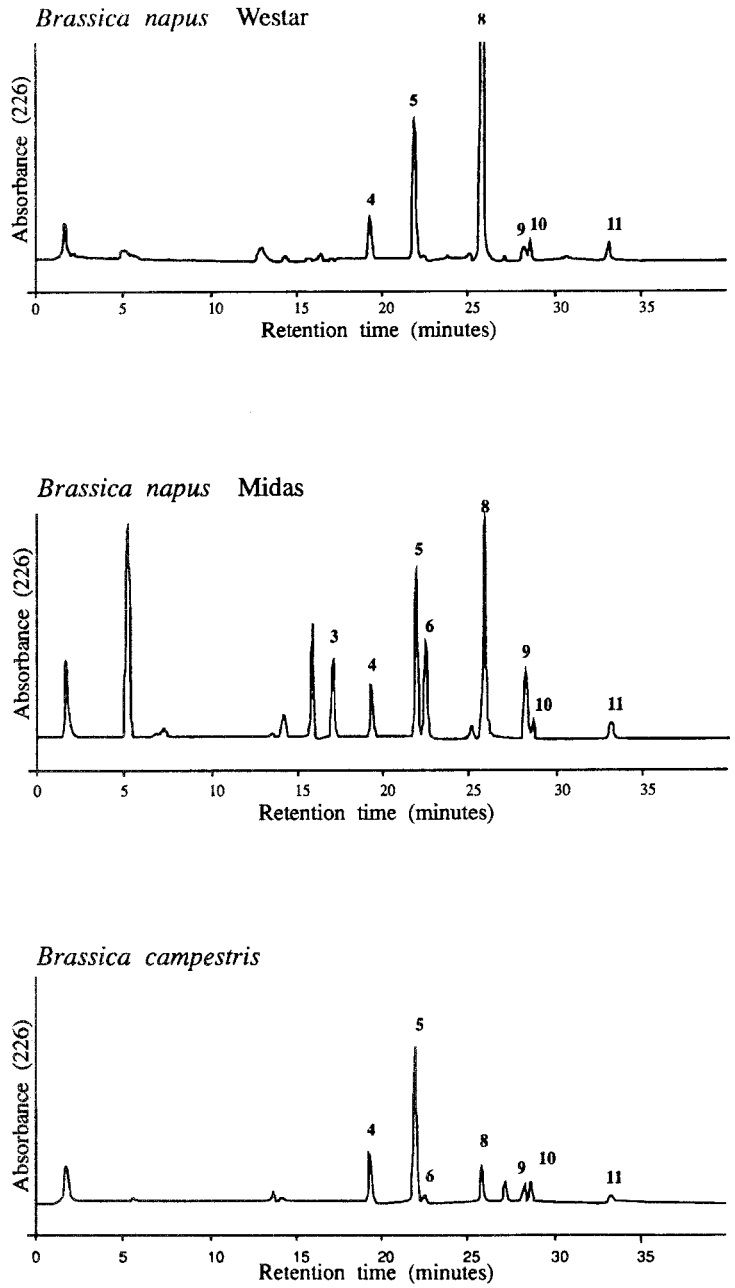
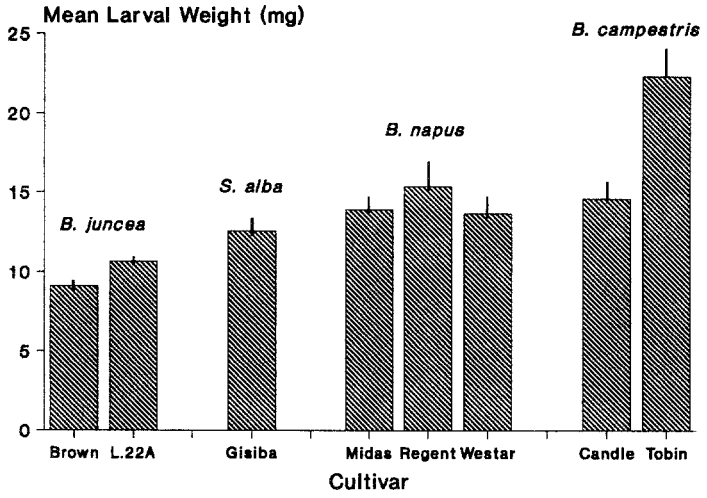


FIG. 2. Representative HPLC chromatograms of glucosinolate profiles for rape species used in the study. Peak numbers as in Figure 1.



I.D.F. Tests

Contrast	F	Pr>F
B. juncea vs others	35.57	.0001
S. alba vs *B. napus* + *B. campestris*	7.72	.0058
B. napus vs *B. campestris*	13.99	.0002
B. juncea: Brown vs Lethbridge 22A	0.96	.3288
B. napus: Midas vs Regent + Westar	0.10	.7526
B. napus: Regent vs Westar	0.89	.3458
B. campestris: Candle vs Tobin	21.63	.0001

FIG. 3. Mean final weights of *M. configurata* larvae reared for seven days on intact rosette-stage plants. Vertical lines above bars represent SEM.

Acceptability to neonates was not correlated to foliar concentrations of total glucosinolates (Table 2). In general, the isothiocyanate-releasing glucosinolates had a negative influence on acceptability, while the thiocyanate-releasing glucosinolates had a positive influence. The influence of glucosinolates on acceptability to neonates was more obvious with stage 3 and stage 4 foliage than with rosette foliage. For stage 3 plants, the numbers of larvae attracted to the leaf disks was negatively correlated to the isothiocyanate-releasing glucosinolate concentration in the foliage, and this was consistent over both blocks of the experiment.

The consumption and growth rates of fourth-instar *M. configurata* were

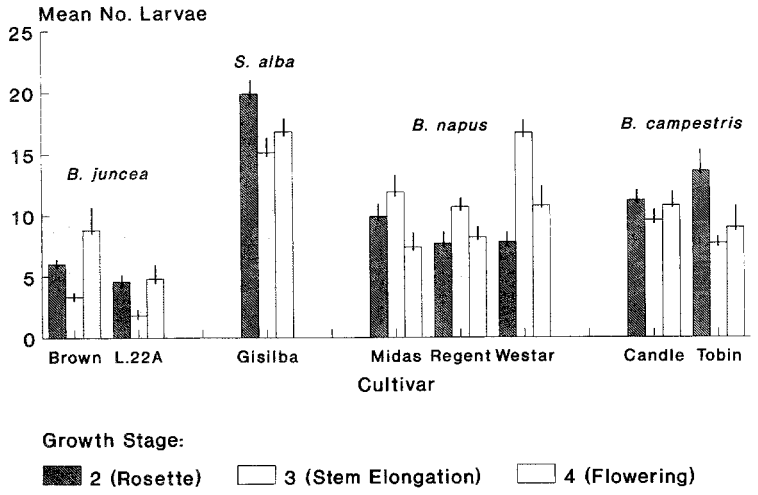


FIG. 4. Numbers of neonate *M. configurata* on leaf disks in choice tests with field-grown foliage, presented as weighted means of square-root transformed data. Vertical lines above bars represent SEM.

TABLE 1. EFFECT OF HOST SPECIES AND CULTIVAR ON NEONATE *M. configurata* HOST SELECTION (BASED ON LEAF DISC CHOICE TESTS), AND RELATIVE CONSUMPTION RATE (RCRi), AND RELATIVE GROWTH RATE (RGRi) OF FOURTH INSTAR LARVAE AS DETERMINED BY ANOVA AND ORTHOGONAL CONTRASTS

	Growth stage								
	Host choice			RCRi			RGRi		
Contrast	2	3	4	2	3	4	2	3	4
B. juncea vs. others	**[a]	**	*	nsd	**	*	nsd	**	**
S. alba vs. *B. napus* + *B. campestris*	**	*	**	nsd	**	nsd	nsd	nsd	nsd
B. napus vs. *B. campestris*	**	**	nsd	nsd	nsd	nsd	nsd	nsd	nsd
B. juncea: Brown vs. Lethbridge 22A	nsd	nsd	nsd	*	nsd	nsd	nsd	nsd	nsd
B. napus									
Midas vs. Regent + Westar	nsd	nsd	nsd	*	nsd	nsd	nsd	*	nsd
Regent vs. Westar	nsd	**	nsd	nsd	nsd	nsd	nsd	nsd	*
B. campestris: Candle vs. Tobin	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd

[a] * = significant at $P < 0.05$; ** = significant at $P < 0.01$; nsd = no significant difference.

TABLE 2. LINEAR CORRELATION COEFFICIENTS FOR RELATIONSHIP OF FOLIAR GLUCOSINOLATES (μmol/g dry wt) TO *M. configurata* NEONATE CHOICE (WEIGHTED MEANS OF SQUARE ROOT-TRANSFORMED COUNTS) AND TO RELATIVE CONSUMPTION RATE (RCRi) AND RELATIVE GROWTH RATE (RGRi) OF FOURTH-NSTAR LARVAE

	Neonate host choice			Fourth-instar RCRi			Fourth-instar RGRi		
Growth stage	Total	ITC type[a]	SCN type[b]	Total	ITC type	SCN type	Total	ITC type	SCN type
2, Rosette									
Block 1	$r = -0.300$	$r = -0.409$	$r = 0.840$	$r = -0.611$	$r = -0.642$	$r = 0.123$	$r = -0.909$	$r = -0.60$	$r = -0.032$
	$P = 0.470$	$P = 0.314$	$P = 0.009$	$P = 0.108$	$P = 0.086$	$P = 0.773$	$P = 0.002$	$P = 0.116$	$P = 0.953$
Block 2	$r = 0.063$	$r = -0.524$	$r = 0.476$	$r = -0.669$	$r = 0$	$r = 0.176$	$r = 0$	$r = 0.095$	$r = -0.434$
	$P = 0.893$	$P = 0.183$	$P = 0.232$	$P = 0.100$	$P = 0.966$	$P = 0.675$	$P = 0.991$	$P = 0.827$	$P = 0.284$
3, Stem elongation									
Block 1	$r = -0.557$	$r = -0.787$	$r = 0.634$	$r = -0.580$	$r = -0.266$	$r = -0.804$	$r = -0.817$	$r = -0.887$	$r = 0.253$
	$P = 0.151$	$P = 0.020$	$P = 0.091$	$P = 0.132$	$P = 0.524$	$P = 0.016$	$P = 0.013$	$P = 0.003$	$P = 0.545$
Block 2	$r = -0.692$	$r = -0.766$	$r = 0.383$	$r = -0.840$	$r = -0.75$	$r = -0.173$	$r = -0.857$	$r = -0.778$	$r = -0.063$
	$P = 0.057$	$P = 0.027$	$P = 0.348$	$P = 0.009$	$P = 0.032$	$P = 0.682$	$P = 0.007$	$P = 0.023$	$P = 0.886$
4, Flowering									
Block 1	$r = -0.265$	$r = -0.533$	$r = 0.801$	$r = -0.574$	$r = -0.530$	$r = 0.207$	$r = -0.807$	$r = -0.743$	$r = 0.145$
	$P = 0.528$	$P = 0.174$	$P = 0.017$	$P = 0.136$	$P = 0.176$	$P = 0.620$	$P = 0.016$	$P = 0.035$	$P = 0.731$

[a] Isothiocyanate (ITC) releasing glucosinolates.
[b] Thiocyanate-releasing glucosinolates.

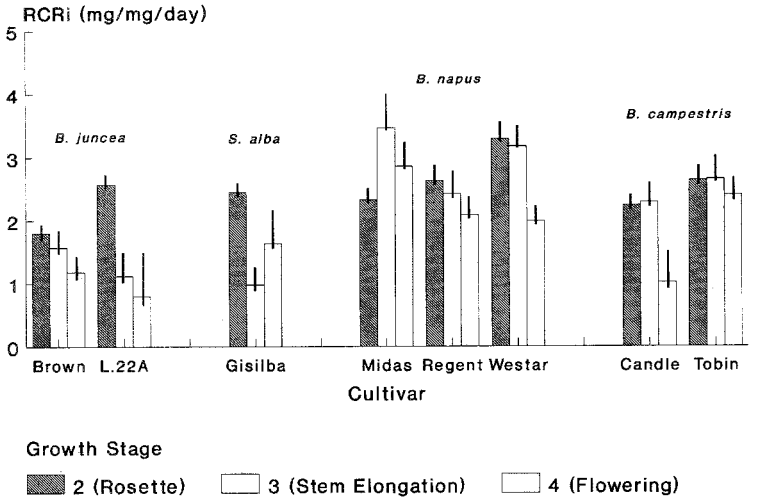


FIG. 5. Mean consumption indices for fourth-instar *M. configurata* larvae on field-grown foliage. Vertical lines above bars represent SEM.

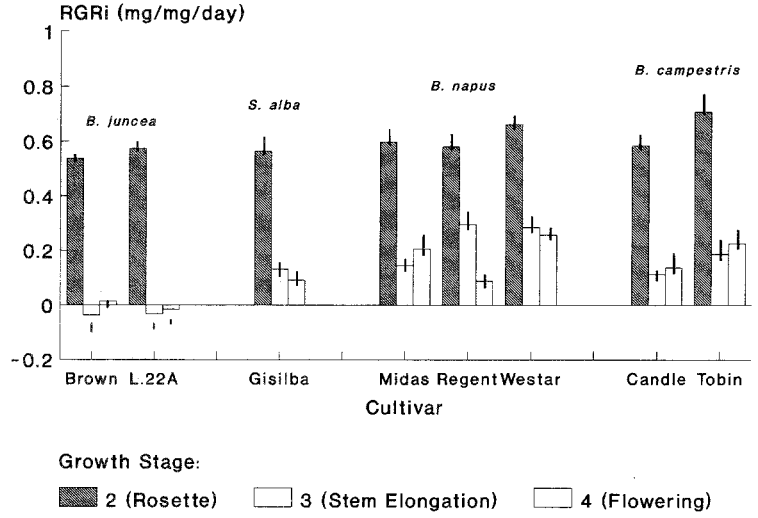


FIG. 6. Mean relative growth rates of fourth-instar *M. configurata* larvae on field-grown foliage. Vertical lines above bars represent SEM.

also influenced by the host species and cultivar (Figures 5 and 6). Predictably, lower consumption rates (RCRi) were observed on *B. juncea* than on the other host plants (Table 1), even though the difference was of borderline significance ($P = 0.0552$) for rosette stage foliage. However, the attractiveness of *S. alba* observed in the neonate choice tests was not reflected by the fourth-instar consumption rates. In fact, the mean RCRi on *S. alba* was significantly lower than those on *B. napus* and *B. campestris* stage 3 foliage. There were no significant differences in mean RCRi between *B. napus* and *B. campestris* for foliage of any growth stage.

Relative growth rates (RGRi) of fourth-instar larvae were reduced on *B. juncea* compared to the other plant species for stage 3 and 4 foliage, and these differences were highly significant (Table 1). However, no significant differences were detected for the rosette stage foliage in any of the contrasts. *S. alba* did not appear to inhibit the growth of fourth-instar larvae as observed with neonates. The comparison of *B. napus* with *B. campestris* also produced no significant differences, although with stage 3 foliage the growth rate of larvae on *B. napus* was somewhat reduced compared to that on *B. campestris* ($P = 0.06$). This was most likely due to the influence of the high-glucosinolate *B. napus* cv. Midas, which yielded a significantly reduced RGRi compared with the low-glucosinolate cultivars Regent and Westar.

These effects on fourth-instar *M. configurata* can be linked to the glucosinolate composition of the plants in some cases. However, a relationship between RCRi and glucosinolate content is not well-defined. Consumption rate tends to be inversely related to the concentration of total glucosinolates, but there was no significant linear correlation except in the second block of the stem elongation stage foliage (Table 2). Simple linear correlations between RCRi and isothiocyanate- and thiocyanate-releasing glucosinolates were also equivocal.

The relationship of RGRi and glucosinolate content appears to be relatively straightforward. RGRi is significantly negatively correlated to total glucosinolate content in the more mature plant growth stages, although the results are inconsistent in the rosette stage (Table 2). These correlations appear to be mainly due to the concentration of isothiocyanate-releasing glucosinolates, as RGRi is also significantly negatively correlated to the levels of these glucosinolates, but not to thiocyanate-releasing glucosinolates.

The data generated by feeding studies using intact plant tissue is partially supported by the results of the feeding study using pure compounds in meridic diet (Figure 7). The negative growth responses of neonate *M. configurata* larvae to equimolar concentrations of sinigrin and its metabolite, allyl isothiocyanate are similar, in that significant linear relationships between larval weight and dietary allelochemical concentration were obtained. However, allyl isothiocyanate in the diets resulted in abnormally high mortality rates, which was partially due to the fumigation effect of this volatile compound (unpublished data). No

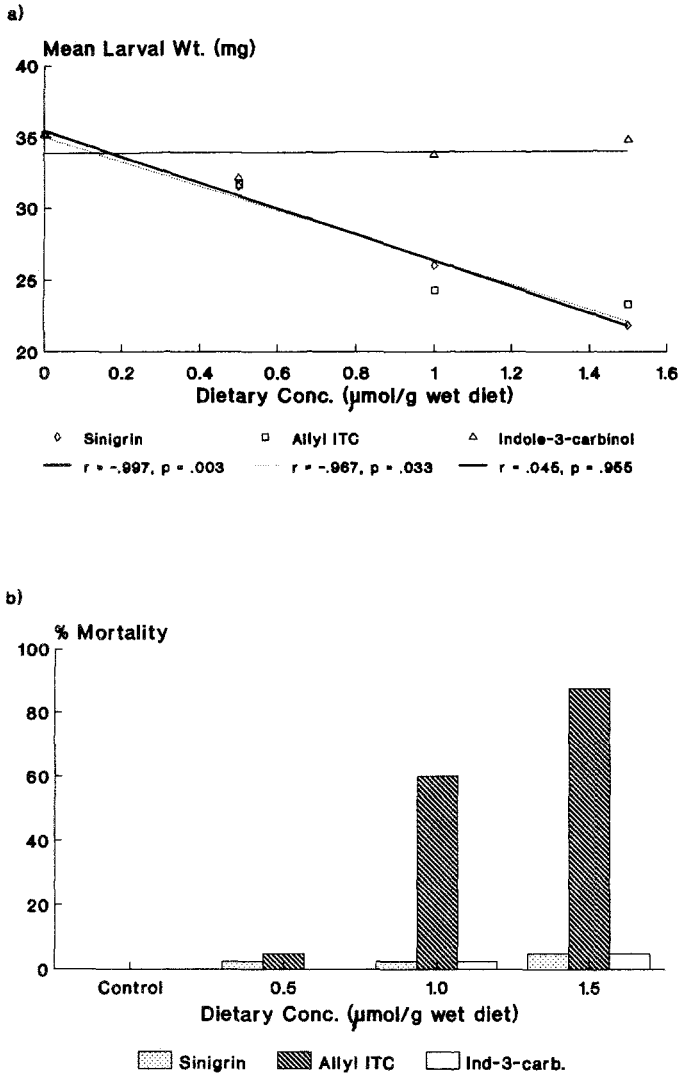


FIG. 7. Results of eight-day feeding assay with pure compounds augmented to meridic diet: (a) linear regression of final weight versus dietary concentration; (b) percent mortality versus dietary concentration.

such growth or mortality responses were observed with indole-3-carbinol, the degradation product of 3-indolylmethyl glucosinolate, which suggests that indolyl alcohols are not biologically active at the concentrations tested.

DISCUSSION

Our feeding studies using artificial diets and various rapes and mustards have established clearly that sinigrin and its metabolite, allyl isothiocyanate, adversely affect the growth of neonate and fourth-instar *M. configurata* larvae. These effects may be due in part to reduced rates of feeding on substrates that contain sinigrin. *B. juncea*, which contains very high levels of sinigrin, is relatively resistant to this polyphagous insect. This mustard was also found to be less preferred by the flea beetle, *Phyllotreta striolata*, than *B. oleracea*, *B. napus*, and *B. campestris* (Lamb and Palaniswamy, 1990). Currently, *B. juncea* is being considered for development as an oilseed because of its superior agronomic performance compared to the current canola species (Woods et al., 1991). The expression of high levels of sinigrin in the foliage may be viewed as another desirable trait of this mustard species.

The effects of thiocyanate-releasing glucosinolates are not as well defined as the effects of isothiocyanate-releasing glucosinolates. The artificial diet test of indole-3-carbinol suggests that this type of glucosinolate metabolite is relatively innocuous to neonate *M. configurata* larvae. However, the leaf disk choice tests suggest that OH-benzyl glucosinolate (sinalbin) may be a feeding stimulant for neonate larvae. Yet, larval growth on intact *S. alba* plants, which produce predominantly sinalbin, was relatively inhibited. Sinalbin previously has been shown to be a factor of antixenotic and antibiotic resistance to Bertha armyworm and flea beetles (Bodnaryk, 1991). However, it should be noted that benzyl glucosinolate occurs in significant amounts (0.7–3.9 μmol/g dry wt, depending on growth stage) in *S. alba* cv. Gisilba. Benzyl isothiocyanate has been shown to be toxic to European corn borer and fall armyworm (Bartlet and Mikolajczak, 1989). It is possible that benzyl glucosinolate may be partially responsible for the growth inhibitory effects observed with *M. configurata* on intact plants. The feeding stimulant effect we observed with *S. alba* in the neonate choice test may simply be due to the presence of relatively high glucoside concentrations with lower levels of associated isothiocyanates.

Thiocyanate-releasing glucosinolates in foliage do not appear to influence consumption by fourth-instar *M. configurata*. Rather, their relative consumption rate is actually reduced on stage 3 foliage of *S. alba*. Since there was no corresponding reduction in larval growth rate attributable to thiocyanate-releasing glucosinolates, it is probable that other factors also affect the consumption of foliage. In the Brassicaceae, glucosinolates are the dominating group of sec-

ondary plant substances and must be considered in questions of insect–plant interactions. However, substrate texture, nutrients, and other types of allelochemicals can also affect the response of insects to these plants. The data from this study suggest that plant growth stage can modify the relative expression of allelochemical effects. This will be the subject of a future paper.

Presently, rapeseed plant breeding efforts are directed towards eliminating glucosinolates due to their antinutritional effects on livestock that consume the seed meal. Although the canola cultivars are nutritionally superior to the mustards in this regard, our study suggests that isothiocyanate-releasing glucosinolates in the foliage may benefit the crop by providing a degree of protection from polyphagous insects like the Bertha armyworm. To this end, a useful target for genetic engineering may be the expression of isothiocyanate-releasing glucosinolates specifically in the foliage coupled with the elimination of glucosinolates from the seed.

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