The role of spatial scale and intraspecific variation in secondary chemistry in host-plant location by *Ceutorhynchus assimilis* (Coleoptera: Curculionidae)

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To understand the ecological role of secondary plant compounds in host location by phytophagous insects it is important to consider attraction at different scales in natural populations. The cabbage seed weevil, *Ceutorhynchus assimilis*, which lays eggs in pods of crucifers where the larvae feed on seed, is attracted to purified extracts of specific glucosinolate-derived volatiles. We considered the possibility that *C. assimilis* adults are attracted to and preferentially attack patches of plants and/or individual plants producing these volatiles. Using discrete natural populations of *Brassica oleracea* and *Brassica nigra*, we found that oviposition was highest in populations of *B. oleracea* producing high amounts of 3-butenylglucosinolate. No links were found between the other glucosinolates, 2-propenylglucosinolate, 2-hydroxy-3-butenylglucosinolate, 1-indolylmethylglucosinolate or 1-methoxy-3-indolylmethylglucosinolate, and oviposition in *B. oleracea*. *B. nigra*, which contains only 2-propenylglucosinolate, was not attacked by *C. assimilis*. Within populations of *B. oleracea*, neither oviposition nor the number of seeds eaten was related to the glucosinolate profiles of individual plants. We suggest that *C. assimilis* adults use 3-butenylglucosinolate-derived volatiles to locate host populations, whereas other cues determine oviposition on individual plants. The consequences of these results for natural selection of glucosinolate phenotypes are discussed.

Keywords: glucosinolates; *Brassica oleracea*; cabbage seed weevil; plant volatiles; attractant

1. INTRODUCTION

Herbivorous insects often specialize on a limited range of host plants, and it is assumed that these specialists commonly use plant-derived volatiles to locate their hosts. Laboratory studies using purified chemicals have shown that some specialist herbivores are attracted to specific plant secondary compounds. However, studies of this effect have not considered attraction to plants in natural communities or the scale over which it acts. Clearly, it is vital that attraction to plants in controlled conditions, or to isolated chemicals, is confirmed in the field before we can understand the ecological role of these chemicals and make inferences about their evolution. Furthermore, the scale over which an attraction occurs will affect the impact it has on, for example, selection pressures on plant secondary compounds.

Glucosinolates are sulphur- and nitrogen-containing secondary metabolites found in the Cruciferae. They are compartmentalized within the cell and when cells are damaged the glucosinolates come into contact with the enzyme myrosinase and a number of products are released. These include volatile nitriles and isothiocyanates, which have been detected in the headspace of brassicas (Tollsten & Bergstrom 1988). There is a large body of evidence that glucosinolates and their breakdown products play a role in determining host specificity for specialist crucifer herbivores (reviewed in Chew 1988; Louda & Mole 1991; Mithen 1992). However, evidence

confirming that these compounds mediate plant–herbivore interactions in natural populations is lacking.

The cabbage seed weevil, *Ceutorhynchus assimilis*, is a specialist on crucifers. The weevil lays eggs in pods (one per pod), where the larvae remain and feed on the seed. Adult weevils show strong electroantennogram responses to 3-butenylisothiocyanate and weaker responses to 2-propenylisothiocyanate (Evans & Allen-Williams 1992). Specific olfactory cells have been located for 3-butenylisothiocyanate (Blight *et al.* 1995). Adult weevils move towards 3-butenylisothiocyanate and isothiocyanate mixtures in controlled conditions (Bartlett *et al.* 1993, 1997; Evans & Allen-Williams 1998) and are attracted to traps baited with these compounds in the field (Smart *et al.* 1997; Smart & Blight 1997). What is not known is whether *C. assimilis* uses 3-butenylisothiocyanate to locate groups of host plants and/or to select individuals within groups. In particular, the role of isothiocyanates in host location in natural plant populations has not been studied. Furthermore, it is not known whether the attraction of adults will lead to oviposition in the pods and herbivory of the seed by the larvae.

Indolyl and aliphatic glucosinolates have been identified in natural populations of *Brassica oleracea* (wild cabbage) and *Brassica nigra* (black mustard) (Mithen *et al.* 1995; Moyes *et al.* 2000). Only 2-propenylglucosinolate has been detected in *B. nigra*, but differences in glucosinolate profiles, including the presence or absence of 3-butenylglucosinolate, have been detected within and between *B. oleracea* populations on the Dorset coast (figure 1). *C. assimilis* is common and causes significant seed loss in these populations (Moyes & Raybould 1998). Thus, this weevil in these populations provides an

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Figure 1. Positions and aliphatic glucosinolate contents ($\mu\text{g/g}$ dry weight) of *Brassica oleracea* populations growing on the Dorset coast. Filled bars denote 2-propenylglucosinolate, hatched bars denote 3-butenylglucosinolate and open bars denote 2-hydroxy-3-butenylglucosinolate. Data from Moyes *et al.* (2000).

excellent system in which to study the role of glucosinolates in host location and herbivory in natural communities.

2. MATERIAL AND METHODS

(a) *Plant populations*

Four discrete wild cabbage populations were used in this study (figure 1). At Durdle Door, Kimmeridge and St Aldhelm's Head, two transects of 50 marked plants were set up in contrasting habitats. Transects were named DD1 and DD2, K1 and K2, and SA1 and SA2, respectively. At Handfast Point one transect (HP) of 50 marked plants was set up. The population sites and transects are described in Moyes *et al.* (2000). Plant density was measured at each site by measuring the distance to the three closest conspecifics for each marked plant. This was not possible at Handfast Point, as plants on the cliff face were not accessible. Not all of the plants flowered in each year, so fewer than 50 plants were used in each analysis. Because some of the plants flowered in both years while others flowered in only one, the data from the two years were neither completely independent nor fully repeated. Therefore, each year was treated separately in all the analyses. Transect K1 was not fully surveyed in 1996 due to landslides.

B. nigra was sampled among and alongside the *B. oleracea* plants at Kimmeridge and St Aldhelm's Head. Ten and 11 plants, respectively, were sampled at each site and a total of 512 pods were examined (see §2b).

(b) *Herbivores and herbivory*

The presence or absence of adult weevils was recorded in all open flowers on five racemes of each plant throughout the flowering seasons of 1995 and 1996.

Herbivory was measured as oviposition rate (proportion of pods infested). Up to ten mature pods were taken from each plant when numbers permitted. For each pod, the length, seed number, number of seeds eaten, larval presence and larval exit holes were recorded. Parasitized larvae, parasite pupae and/or adult parasites in pods were also recorded. Differences in the proportions of pods infested (oviposition rate) between sites

were tested using χ^2 tests, with sequential Bonferroni adjustment ($\alpha=0.05$) for individual comparisons.

One-way analysis of variance (ANOVA) was used to compare the number of adults per flower and the number of seeds eaten per weevil across transects. Individual differences were tested using Tukey's method ($\alpha=0.05$).

(c) *Herbivore choice of glucosinolate profiles*

Details of the range of concentrations of individual glucosinolates in these plants, measured using high performance liquid chromatography–mass spectrometry, have been published previously (Moyes *et al.* 2000) and were measured during the period of this study. The glucosinolates found were 2-propenylglucosinolate, 3-butenylglucosinolate, 2-hydroxy-3-butenylglucosinolate, 1-indolylmethylglucosinolate and 1-methoxy-3-indolylmethylglucosinolate. The two indolylglucosinolates were present in all plants. Glucosinolate profiles varied between individual plants and between populations but not between contrasting habitats within populations.

The effect of the presence of each aliphatic glucosinolate in each plant on herbivory by *C. assimilis* was tested using a generalized linear model (GLM) for each year. Plant size (in terms of raceme number) and mean pod size were used as covariates and each glucosinolate presence and transect were used as factors, with oviposition rate or number of seeds eaten per pod as the response.

The effect of the glucosinolate concentrations of each plant (Moyes *et al.* 2000) on herbivory was tested using a GLM for each year. Plant size (in terms of raceme number), mean pod size and each glucosinolate concentration (aliphatics and indolyls) were used as covariates and transect was used as a factor, with oviposition rate or number of seeds eaten per pod as the response.

(d) *Patch selection*

Regressions were used to compare levels of infestation at the plant-population level with other population factors. The proportion of pods infested per plant and the number of seeds eaten per plant were compared to the mean plant aggregation, the mean proportion of weevils parasitized per plant and the mean concentration of each glucosinolate.

Table 1. Mean numbers of adult weevils found in flowers of marked plants.

(Means $\pm$ s.e.m. across transects are taken from the mean number per flower per plant. N is the number of flowering plants in each transect. Within a column, means that are followed by the same letter are not significantly different from each other (Tukey, $\alpha=0.05$). The overall proportion of pods infested in each population is estimated from up to ten pods from each flowering plant. Proportions followed by the same letter are not significantly different from each other (χ^2 with sequential Bonferroni correction, $\alpha=0.05$).)

transect	1995				1996			
	N	adults per flower	n	proportion of pods infested	N	adults per flower	n	proportion of pods infested
DD1	14	0.36 $\pm$ 0.23a	56	0.16ab	23	0.57 $\pm$ 0.24a	199	0.17a
DD2	14	3.71 $\pm$ 1.02a	77	0.18ab	29	2.79 $\pm$ 0.72b	186	0.18a
HP	25	1.32 $\pm$ 0.48a	110	0.17ab	37	1.14 $\pm$ 0.46a	324	0.18a
K1	9	0.56 $\pm$ 0.56a	44	0.00a	not studied		216	0.06b
K2	27	2.93 $\pm$ 0.64a	127	0.06a	36	0.81 $\pm$ 0.32a	302	0.06b
SA1	33	2.18 $\pm$ 1.54a	165	0.33bc	43	0.28 $\pm$ 0.15a	426	0.38c
SA2	17	0.41 $\pm$ 0.41a	92	0.40c	26	0.19 $\pm$ 0.19a	163	0.36c

3. RESULTS

(a) *Herbivores and herbivory*

There were no significant differences between *B. oleracea* populations in the abundance of adult seed weevils during flowering in 1995. However, in 1996 there were significant differences ($F_{5,188}=5.62$, $p<0.001$), mainly due to the greater numbers of adult weevils recorded at DD2 (table 1). In both years, there were significant differences between plant populations in the proportion of pods infested by the weevil larvae (1995, $\chi^2_6=65.58$, $p<0.001$; 1996, $\chi^2_6=174.23$, $p=0.001$), but there were no significant differences between habitats within populations (table 1). There were no significant differences in the numbers of seeds eaten per larva between transects (1995, $F_{6,664}=1.304$, $p=0.253$; 1996, $F_{6,1809}=0.494$, $p=0.813$), and there was a close relationship between oviposition rate per plant and the number of seeds eaten per plant (1995, $r^2=0.86$, $p<0.001$; 1996, $r^2=0.87$, $p<0.001$).

No weevils were found in pods of *B. nigra*, so the following sections refer to *B. oleracea* alone.

(b) *Herbivore choice of individual plants*

Within plant populations, no links were found between the oviposition rate or the number of seeds eaten on a particular plant and the presence or concentration of any glucosinolate (Appendix A). Figure 2 shows the relationship between oviposition per plant and 3-butenylglucosinolate as a typical example. Thus, the glucosinolate profile did not influence either the likelihood of attack or the level of herbivory by individual larvae. Neither response was linked to plant size but both were linked to pod size in both years (Appendix A).

(c) *Herbivore choice of plant patches*

In both years there was a strong positive link between the *C. assimilis* oviposition rate per plant population and the 3-butenylglucosinolate content of that plant population (1995, $r^2=0.97$, $p=0.016$; 1996, $r^2=0.97$, $p=0.015$; figure 3). There was also a positive correlation between the 3-butenylglucosinolate content and the number of

seeds eaten per plant (1995, $r^2=0.82$, $p=0.097$; 1996, $r^2=0.95$, $p=0.030$).

No links were found between the oviposition rate per plant population and either plant aggregation (1995, $r^2=0.02$, $p=0.91$; 1996, $r^2<0.01$, $p=0.99$) or parasitism (1995, $r^2=0.02$, $p=0.86$; 1996, $r^2=0.28$, $p=0.47$). In addition, we did not detect any relationship between the oviposition rate per plant population and the mean content of any other glucosinolate (propenylglucosinolate: 1995, $r^2<0.01$, $p=0.99$; 1996, $r^2=0.09$, $p=0.70$; hydroxybutenylglucosinolate: 1995, $r^2=0.01$, $p=0.91$; 1996, $r^2=0.05$, $p=0.77$; indolylmethylglucosinolate: 1995, $r^2=0.11$, $p=0.67$; 1996, $r^2=0.47$, $p=0.32$; methoxyindolylmethylglucosinolate: 1995, $r^2=0.43$, $p=0.35$; 1996, $r^2=0.32$, $p=0.43$). Similarly, we found no relationship between the number of seeds eaten per plant and plant aggregation (1995, $r^2=0.16$, $p=0.74$; 1996, $r^2=0.01$, $p=0.93$), parasitism (1995, $r^2=0.15$, $p=0.61$; 1996, $r^2=0.25$, $p=0.50$), or any other glucosinolates (propenylglucosinolate: 1995, $r^2=0.03$, $p=0.82$; 1996, $r^2=0.15$, $p=0.61$; hydroxybutenylglucosinolate: 1995, $r^2=0.02$, $p=0.86$; 1996, $r^2=0.02$, $p=0.86$; indolylmethylglucosinolate: 1995, $r^2=0.01$, $p=0.90$; 1996, $r^2=0.38$, $p=0.38$; methoxyindolylmethylglucosinolate: 1995, $r^2=0.56$, $p=0.25$; 1996, $r^2=0.39$, $p=0.38$).

4. DISCUSSION

Within plant populations, oviposition on individual plants was not linked to their glucosinolate profiles and glucosinolate profiles did not influence the number of seeds eaten following oviposition.

The pattern of variation in oviposition between plant populations did not reflect the pattern of abundance of adult weevils, but did match that of the glucosinolates, which varied between plant populations but not between habitats within populations (Moyes *et al.* 2000). The oviposition rate was greatest in plant populations containing the highest mean amounts of 3-butenylglucosinolate. The number of seeds eaten per plant was also closely related to the mean amount of 3-butenylglucosinolate in the population.

The breakdown products of glucosinolates, isothiocyanates and nitriles, have been detected in the headspaces

Figure 2. Comparisons of 3-butenylglucosinolate content and pod infestation in individual plants in each plant population. Diamonds represent transect 1 and circles represent transect 2. Open symbols represent 1995 data and filled symbols represent 1996 data. See Appendix A for statistical tests of the effects of the presence or absence and content of the individual glucosinolates.

of *Brassica* species when plants were damaged (Tollsten & Bergstrom 1988), which is a frequent occurrence in these populations growing in exposed coastal environments (Moyes 1997). Compounds released into the atmosphere

Figure 3. Comparisons of population means of 3-butenylglucosinolate content and pod infestation in the two years. Diamonds represent 1995 data and squares represent 1996 data.

around a plant may not be sufficiently localized to allow herbivores to distinguish between individual plants. If *C. assimilis* responds to glucosinolate breakdown products in the atmosphere, but not to the intact glucosinolates in the plant, this may explain why no selection of individual plants was found.

The discrepancy between the numbers of adults feeding in flowers and the proportion of pods attacked in each population may be explained by the behaviour of this species, which has been well documented in studies of crop pests (Finch & Thompson 1992; Paul & Rawlinson 1992; Gratwick 1992). Adults emerge in spring and feed locally on flowers and pods causing only small amounts of damage. They are strong fliers and can travel to new patches of plants, where they may release an aggregating pheromone (Evans & Scarisbrick 1994). They travel to crops when temperatures rise to over 13 °C and females oviposit in young pods from late May to mid June. The larvae feed for 4–5 weeks, consuming three to five seeds depending on their size. They then chew a distinctive hole in the pod wall, about 1 mm in diameter, and pupate in the soil. After 2 weeks the adults emerge and move to crops or wild plants to feed. They then migrate to sheltered places such as woods, hedges, leaf litter, the soil and under bark to hibernate.

The first generation is most relevant to our plant populations as they flowered early, from April in 1995 and from early May in 1996, before oilseed-rape crops flower. The adults we observed may have emerged and fed prior to migrating to new areas. To study this further, adults would need to be monitored throughout both flowering and pod development.

Three mechanisms could account for the discrepancy between adult and larval numbers. First, adults may be less likely to emigrate from an area where high levels of 3-butenylisothiocyanate are present. Second, adults may be more likely to migrate into areas where high levels of 3-butenylisothiocyanate are present. Third, adults may be less likely to oviposit in the absence of, or when exposed to low levels of, 3-butenylglucosinolate.

Within populations, plants containing no 3-butenylglucosinolate were equally likely to be attacked as those producing this compound. Furthermore, the glucosinolate concentration of plants fed on by adult weevils does not influence

oogenesis (Ni *et al.* 1990). Thus, it is more likely that one or both of the first two mechanisms account for our results.

Larvae feeding in populations with low levels of 3-butenylglucosinolate ate as many seed and had the same probability of surviving as those elsewhere. Parasitism was no greater in these plant populations (Moyes & Raybould 1998) and pod availability was not limited. Therefore, these plant populations appear to offer resources that are as good as high-3-butenylglucosinolate populations. If adults migrate out of suitable habitat, they run the risk of being unable to find a new resource habitat (Haccou *et al.* 1999; Loxdale & Lushai 1999), although studies of flea beetles in patches of *B. oleracea* found that their densities were influenced more by emigration than by immigration (Kareiva 1985). If the effect we have observed is due to differential immigration, further work needs to be carried out to elucidate more precisely the scale over which attraction to isothiocyanates occurs. Unfortunately, we found no significant differences in 3-butenylglucosinolate quantities between habitats within plant populations, so study of attraction at this scale was not possible. However, it has been shown that adult weevils can locate *Brassica napus* odours released from traps at a distance of at least 20 m (Evans & Allen-Williams 1993).

The use of plant patches set up in a controlled design would allow the scale of attraction to be studied further. Although our study was, in some ways, limited by the use of natural populations, it does not suffer from the problems of extrapolating from field plots to ecological situations (Banks 1998). Care needs to be taken in interpreting these results as other factors may vary between these populations, which, if correlated with 3-butenylglucosinolate levels, could confound the results presented here. However, there is strong corroboration from laboratory studies that 3-butenylisothiocyanate plays a role in determining host location by the weevil. Controlled experiments have shown that *C. assimilis* is most attracted to 3-butenylisothiocyanate, of the isothiocyanates tested (Evans & Allen-Williams 1992, 1998; Blight *et al.* 1995; Bartlett *et al.* 1993, 1997), and 3-butenylglucosinolate was the only glucosinolate whose distribution was linked to larval infestation in this study.

In conclusion, our results are consistent with the hypothesis that, prior to ovipositing, *C. assimilis* adults are attracted to areas where plants are releasing large quantities of 3-butenylisothiocyanate, but they are not stimulated to oviposit in individual plants by a particular aliphatic glucosinolate. To test this further, male and female adults should be monitored throughout flowering and pod production; controlled experiments on oviposition in response to glucosinolates would complement those carried out on detection and attraction.

In order to characterize further the scale of this attraction, plant populations need to be manipulated. This would enable determination of the critical distances between neighbouring plants and the quantities of 3-butenylisothiocyanate necessary to attract weevils. With this information it would be possible to discern whether threshold levels exist or whether the response is linear.

It is interesting to speculate on whether the feeding behaviour of the cabbage seed weevil is driving the evolution of glucosinolate profiles in *B. oleracea*. Moyes &

Raybould (1998) showed that *B. oleracea* plants infested with weevils produce significantly fewer seeds than uninfested plants. However, this study has shown that the individual glucosinolate profile does not determine the extent to which the plant is infested by the weevil; the amount of herbivory is determined by the average glucosinolate profile of a group of neighbouring plants. This suggests that the weevil cannot affect the distribution of glucosinolates in these populations.

However, *B. oleracea* populations in Dorset are highly structured because seed and pollen dispersal are very limited (Raybould *et al.* 1999), and the glucosinolate profiles of these populations are under simple genetic control (Mithen *et al.* 1995). A plant will produce high levels of 3-butenylglucosinolate relative to 2-propenylglucosinolate and 2-hydroxy-3-butenylglucosinolate if it has a functional (or 'fast') allele at locus *gsl-elong* and is homozygous for null (or 'slow') alleles at locus *gsl-oh*. Therefore, populations could be structured into homogeneous patches of plants with high and low levels of 3-butenylglucosinolate, which could be selected by the weevil. Such selection would favour the spread of patches of plants with a high frequency of *gsl-elong*—and/or a low frequency of *gsl-oh*+. To maintain the polymorphism, under certain circumstances there must be a selective advantage to plants with high levels of 3-butenylglucosinolate, for example in deterring herbivory by slugs (Giamoustaris & Mithen 1995). Of course, it is possible that the distribution of glucosinolate variation is due entirely to restricted gene flow and genetic drift, and that the prevalence of the weevil tracks this distribution without affecting it. Reciprocal-transplant experiments may help to determine whether selection or drift is the main factor determining glucosinolate variation in these populations.

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APPENDIX A

ANOVA tables for tests of the effects of the presence or absence and concentration of individual aliphatic

Table A1. ANOVA testing the effect of individual glucosinolate presence on oviposition rate per plant in 1995.

(Asterisk denotes a covariate.)

source	d.f.	adjSS	MS	F	p
plant size*	1	0.05	0.05	0.82	0.368
pod length*	1	0.20	0.20	3.39	0.069
transect	6	0.44	0.07	1.25	0.290
hydroxybutenylglucosinolate	1	< 0.01	< 0.01	< 0.01	0.949
propenylglucosinolate	1	0.01	0.01	0.21	0.649
butenylglucosinolate	1	0.10	0.10	1.61	0.207
error	90	5.39	0.06		
total	101				

Table A2. ANOVA testing the effect of individual glucosinolate presence on oviposition rate per plant in 1996.

(No data were available for transect K1. Asterisk denotes a covariate.)

source	d.f.	adjSS	MS	<i>F</i>	<i>p</i>
plant size*	1	0.01	0.01	0.17	0.678
pod length*	1	0.06	0.06	1.71	0.194
transect	5	1.30	0.26	7.04	< 0.001
hydroxybutenyl-glucosinolate	1	0.07	0.07	1.88	0.173
propenylglucosinolate	1	0.06	0.06	1.54	0.217
butenylglucosinolate	1	0.07	0.07	1.94	0.166
error	117	4.33	0.04		
total	127				

Table A3. ANOVA testing the effect of individual glucosinolate presence on the number of seeds eaten by larvae per plant in 1995.

(Asterisk denotes a covariate.)

source	d.f.	adjSS	MS	<i>F</i>	<i>p</i>
plant size*	1	0.98	0.98	1.27	0.262
pod length*	1	3.03	3.03	3.94	0.05
transect	6	2.00	0.33	0.43	0.854
hydroxybutenyl-glucosinolate	1	0.01	0.01	0.02	0.899
propenylglucosinolate	1	0.28	0.28	0.36	0.549
butenylglucosinolate	1	0.91	0.91	1.18	0.280
error	90	69.15	0.77		
total	101				

Table A4. ANOVA testing the effect of individual glucosinolate presence on the number of seeds eaten by larvae per plant in 1996.

(No data were available for transect K1. Asterisk denotes a covariate.)

source	d.f.	adjSS	MS	<i>F</i>	<i>p</i>
plant size*	1	0.01	0.01	0.04	0.837
pod length*	1	1.59	1.59	5.49	0.021
transect	5	1.91	1.91	6.56	< 0.001
hydroxybutenyl-glucosinolate	1	0.85	0.85	2.94	0.089
propenylglucosinolate	1	0.71	0.71	2.46	0.120
butenylglucosinolate	1	0.43	0.43	1.49	0.225
error	117	33.99	0.29		
total	127				

Table A5. ANOVA testing the effect of individual glucosinolate concentrations on oviposition rate per plant in 1995.

(Asterisk denotes a covariate.)

source	d.f.	adjSS	MS	<i>F</i>	<i>p</i>
plant size*	1	0.05	0.05	0.78	0.380
pod length*	1	0.33	0.33	5.66	0.020
hydroxybutenyl-glucosinolate*	1	0.03	0.03	0.54	0.465
propenylglucosinolate*	1	0.03	0.03	0.49	0.484
butenylglucosinolate*	1	0.16	0.16	2.78	0.099
indolylmethyl-glucosinolate*	1	0.05	0.05	0.89	0.349
methoxyindolylmethyl-glucosinolate*	1	0.01	0.01	0.12	0.727
transect	6	0.33	0.06	0.96	0.457
error	88	5.12	0.06		
total	101				

Table A6. ANOVA testing the effect of individual glucosinolate concentrations on oviposition rate per plant in 1996.

(No data were available for transect K1. Asterisk denotes a covariate.)

source	d.f.	adjSS	MS	<i>F</i>	<i>p</i>
plant size*	1	< 0.01	< 0.01	0.01	0.930
pod length*	1	0.07	0.07	1.87	0.174
hydroxybutenyl-glucosinolate*	1	0.03	0.03	0.74	0.393
propenylglucosinolate*	1	0.10	0.10	2.69	0.103
butenylglucosinolate*	1	< 0.01	< 0.01	0.03	0.871
indolylmethyl-glucosinolate*	1	0.03	0.03	0.93	0.338
methoxyindolylmethyl-glucosinolate*	1	0.02	0.02	0.50	0.482
transect	5	1.08	0.22	5.75	< 0.001
error	115	4.33	0.04		
total	127				

Table A7. ANOVA testing the effect of individual glucosinolate concentrations on the number of seeds eaten by larvae per plant in 1995.

(Asterisk denotes a covariate.)

source	d.f.	adjSS	MS	<i>F</i>	<i>p</i>
plant size*	1	0.80	0.80	1.07	0.303
pod length*	1	4.40	4.40	5.88	0.017
hydroxybutenyl-glucosinolate*	1	0.21	0.21	0.28	0.601
propenylglucosinolate*	1	0.79	0.79	1.06	0.306
butenylglucosinolate*	1	1.08	1.08	1.44	0.233
indolylmethyl-glucosinolate*	1	1.08	1.08	1.45	0.232
methoxyindolylmethyl-glucosinolate*	1	0.15	0.15	0.20	0.654
transect	6	2.64	0.44	0.59	0.740
error	88	65.84	0.75		
total	101				

Table A8. ANOVA testing the effect of individual glucosinolate concentrations on the number of seeds eaten by larvae per plant in 1996.

(No data were available for transect K1. Asterisk denotes a covariate.)

source	d.f.	adjSS	MS	F	p
plant size*	1	< 0.01	< 0.01	0.01	0.921
pod length*	1	1.91	1.91	6.42	0.013
hydroxybutenyl-glucosinolate*	1	0.13	0.13	0.45	0.506
propenylglucosinolate*	1	0.72	0.72	2.42	0.122
butenylglucosinolate*	1	0.07	0.07	0.25	0.618
indolylmethyl-glucosinolate*	1	0.47	0.47	1.57	0.213
methoxyindolylmethyl-glucosinolate*	1	0.27	0.27	0.91	0.342
transect	5	6.22	1.24	4.19	0.002
error	115	34.13	0.30		
total	127				

glucosinolates on oviposition rate and seed herbivory by *C. assimilis* in natural populations of *B. oleracea*.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.