

Combined Effect of Intercropping and Turnip Root Fly (*Delia floralis*) Larval Feeding on the Glucosinolate Concentrations in Cabbage Roots and Foliage

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Abstract The effects of plant competition and herbivory on glucosinolate concentrations in cabbage root and foliage were investigated in a cabbage-red clover intercropping system. Cabbage plants were grown under different competitive pressures and with varying degrees of attack by root-feeding *Delia floralis* larvae. Glucosinolate concentrations in cabbage were affected both by intercropping and by *D. floralis* density. Glucosinolate concentrations in foliage generally decreased as a response to intercropping, while the responses to insect root damage of individual glucosinolates were weaker. Root glucosinolates responded more strongly to both intercropping and egg density. Total root glucosinolate concentration decreased with clover density, but only at high egg densities. Increased egg density led to opposite reactions by the indole and aliphatic glucosinolates in roots. The responses of individual root glucosinolates to competition and root damage were complex and, on occasion, nonlinear. Reduced concentrations of several glucosinolates and the tendency towards a decrease in total concentration in cabbage foliage caused by intercropping and larval damage suggest that competing

plants or plants with root herbivory do not allocate the same resources as unchallenged plants towards sustaining levels of leaf defensive compounds. This could also be true for root glucosinolate concentrations at high egg densities. In addition, the results suggest that changes occurring within a structural group of glucosinolates may be influenced by changes in a single compound, e.g., glucobrassicin (indol-3-ylmethyl) in foliage or sinigrin (2-propenyl) in roots.

Keywords Induced plant responses · Indole glucosinolates · Aliphatic glucosinolates · *Brassica* · Plant–herbivore interactions · Plant competition

Introduction

Plants often respond to abiotic or biotic stress factors with induced changes in their secondary compounds (Yosuf and Collins 1998). In complex systems, plants are likely to be affected by both the stress imposed by their growing environment and attack from different pests that may cause interactive effects in their induced responses. Glucosinolates are the dominant class of secondary compounds present in the family Brassicaceae and constitute approximately 120 different compounds (Halkier and Gershenzon 2006). The content of specific glucosinolates in different brassicaceous plants and their relative concentrations vary among plant species (Ulmer and Dosdall 2006), genotype (Hopkins et al. 1998a), and also depend on plant growth stage and the tissues sampled (Clossais-Besnard and Larher 1991; Hopkins et al. 1998b; Lambdon et al. 2003; Nilsson et al. 2006). When plant tissues are damaged, glucosinolates come into contact with enzymatic myrosinases that catalyze the formation of an array of products, of which the isothiocyanates (mustard oils) are best known. These compounds are

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largely responsible for the characteristic flavors of crucifers. The glucosinolate-myrosinase system is regarded as an important defense strategy for the Brassicaceae as it may protect plants from some generalist pests and pathogens (Giamoustaris and Mithen 1995). However, glucosinolates and their breakdown products also act as stimulants and attractants for insects specialized on brassicaceous hosts (Roessingh et al. 1992; van Loon et al. 1992; Renwick et al. 2006). Many studies to date have concentrated solely on the effect of herbivory on glucosinolate induction in plants. An increase in total glucosinolates, especially those containing an indole-based side chain, has often been observed in plant tissues after herbivore damage (Koritsas et al. 1991; Bartlett et al. 1999; van Dam and Raaijmakers 2006).

In addition to the stress caused by herbivory, other factors also affect glucosinolate concentrations in plants including competition for light, nutrients, and water (Yosuf and Collins 1998; Radovich et al. 2005). While the effects of herbivory have received much attention, little work has been done to establish the effect of plant–plant interactions on the concentrations of secondary compounds in brassicaceous plants; even less effort has been made to investigate the combined effect of herbivory and plant competition. When subjected to herbivory, plants growing in competitive environments may not have access to, or allocate, the same amount of resources towards the synthesis of secondary compounds. Theory predicts that the biological costs of plant defense should increase in environments that expose plants to intense competition (Siemens et al. 2002). The growth-differentiation balance (GDB) hypothesis predicts how plants balance the processes that allocate resources between growth and differentiation depending on environmental conditions (Herms and Mattson 1992). One consequence of this may be that brassicaceous plants grown in highly competitive environments allocate a lower proportion of their resources to the production of defensive compounds, in this case, glucosinolates. Because of reduced attacks from pest insects (Risch 1983; Theunissen 1997) and infestations of specialized insects on brassicaceous plants (Theunissen et al. 1995; Finch and Kienegger 1997; Björkman et al. 2007), intercropping has received increased attention. The main mechanism for pest reduction in intercropped systems is thought to be a disruption of host-plant searching behavior of the herbivore (Finch and Collier 2000) and possibly an enhanced effect of natural enemies suppressing the negative effects of the pest insect (Root 1973). In addition, intercropping often creates an environment where crops have to compete with companion plants for mutual resources. The changes, brought about in the crop plants by the competitive environment, are also likely to affect herbivores in intercropping systems.

It is of interest to investigate how the glucosinolate content of plants is impacted by herbivory in a competitive

environment, such as intercropping, due to the role of these chemicals in plant defense. The present study examined the combined effect of intercropping with red clover and larval feeding by the turnip root fly, *Delia floralis* Fall. (Diptera: Anthomyiidae), on the glucosinolate content of cabbage (*Brassica oleracea* var. *capitata*). The aims were to investigate how the concentration of total and individual glucosinolates in both above and below ground cabbage tissues were affected by (1) competition from different densities of clover, (2) the damage of different densities of *D. floralis*, and (3) whether the co-occurrence of simultaneous stress from clover competition and *D. floralis* herbivory had any additional effects on glucosinolate concentrations.

Methods and Materials

Plant Material Plant material was grown in a greenhouse, under natural light supplemented with 400-W sodium lamps to give an 18:6 h L/D regime. Cabbage (*B. oleracea* var. *capitata* genotype Castello) and red clover (*Trifolium pratense* genotype Betty) were planted in small pots (50×50×50 mm) containing potting soil (Hasselfors Garden AB, Hasselfors, Sweden). Seven-week-old clover plants and 5-week-old cabbage plants were transplanted into plastic boxes (L×W×D=360×255×215 mm) filled with 15 L of a sand-potting soil mixture (one third washed sand – grain size 0.5 to 5 mm and two thirds potting soil).

Insect Material A culture of the turnip root fly, *D. floralis* Fall. (Diptera: Anthomyiidae), was established at the Department of Ecology (Swedish University of Agricultural Sciences) from field-collected pupae [collected in 2002–2003 from an experimental site growing cabbage at Röbbäcksdalen, Umeå (63°45' N; 20°15' E)]. Adult flies were kept at 20°C, 70% RH, 16:8 h L/D regime and fed with a standardized diet of milk, yeast-powder, and honey (based on Finch and Coaker 1969). A Petri dish containing a piece of fresh swede (*Brassica napus* ssp. *rapifera*) was placed on top of moist sand (grain size 1.0–1.6 mm) as an oviposition site. Eggs were floated from the sand with water and sieved through a black cloth. Eggs were taken from the cloth and inoculated onto the plants with a fine paintbrush.

Experimental Procedure Cabbage plants were transplanted into the boxes alone (M) or with one of the two clover densities (IC1 or IC2). Clover plants were transplanted into the boxes at either one row (IC1) of four plants on each side (10 cm from) of the cabbage, or two rows (IC2) of four plants (a total of eight plants) on each side of the cabbage. The boxes were left for 2 weeks in the greenhouse to allow cabbage and clover plants to establish.

The experiment consisted of ten blocks with one block set up each day for ten successive days. Within each block, there were four cabbage plants grown with each clover density (M, IC1, and IC2). Throughout the experiment, the greenhouse was kept at 17°C during the day and 12°C during the night. Light conditions were natural light, supplemented with 400-W sodium lamps when natural light was below 100 W/m², between 6 AM and 9 PM. After 2 weeks of plant establishment *D. floralis* eggs (<24-h old; either 0, 10, 20, or 40) were inoculated on each of the four cabbage plants within the three growing conditions, leading to a total of 12 different treatments within each block. As there were ten blocks, each treatment was replicated ten times. Eggs were inoculated onto the stem base of cabbage plants with a fine brush according to the methods of Birch et al. (1992). At the time of egg inoculation, cabbage plants were at the 6–7 true leaf stage.

Throughout the experiment, only pure water was added to the boxes to create a competitive environment in terms of space, light, and nutrients in the intercropped treatments. Plants were watered according to their requirements, i.e., boxes with clover were given more water so that drought did not influence survival of the turnip root fly larvae. Water was added in moderate doses two times a day. For the first week after egg inoculation, a spray bottle was used to keep the soil near the stem base moist to avoid dehydration of unhatched eggs and to avoid eggs from being washed away from the stem base by ordinary watering. There was no significant difference in the percentage pupation of *D. floralis* depending on the density of eggs applied or the density of clover (Björkman, unpublished data). Consequently, the number of eggs applied is equivalent to herbivore density.

Prior testing of *D. floralis* developmental time under similar conditions revealed that the development time was approximately 6 weeks. Therefore, 6 weeks after egg inoculation, the experiment was terminated at a rate of one block per day. Green cabbage parts were cut to ground level and the cabbage roots were freed from soil. A 6–6.4-g foliage sample and a 1.3–1.7-g root sample were cut from each individual plant and immediately frozen in liquid nitrogen, prior to being lyophilized and milled for glucosinolate analysis. Foliage samples were taken from the youngest leaves, while root samples were taken from the main root. Freeze-dried samples from blocks 1–5 and blocks 6–10 were bulked and analyzed together, with the consequence that each treatment was twice replicated in the chemical analyses.

Glucosinolate Identification Glucosinolate analysis was performed by hplc according to Nilsson et al. (2006). Glucosinolates were identified by their retention times (Rt) and comparison with authentic standards. A rapeseed (*B.*

napus) standard with added sinigrin was used to validate the Rt for all glucosinolates except for glucoraphanin, for which a radish (*Raphanus sativus*) seed sample was used.

Statistical Analyses The overall analysis of glucosinolate content was performed with separate multivariate analyses of variance (MANOVAs) for foliage and root samples. Total glucosinolate concentration, amount of aliphatic or indole glucosinolates, and individual analyses of glucosinolates were carried out with general linear model (GLM). Intercrop and egg densities were used as variables in the model as well as the squared egg density. The latter density was to reveal nonlinear responses to an increase in damage or plant competition. Data for root gluconapin were analyzed by using a log-link to achieve a normal distribution. GLMs were simplified by removing variables that did not improve the explanatory power. This was done by comparing the simplified model with the original model by using an *F* test (Crawley 2003). Variables with a *P* values <0.1 were retained in the model (and are presented in the tables). Nonsignificant variables were excluded as long as they were not included in a significant interaction. Excluded variables are marked with a hatched line in tables, and nonsignificant variables that were retained in the model are marked with N.S. As the number of analyses per root and shoot sample was as many as nine for root and foliage glucosinolates, respectively, a Bonferroni correction would give a corrected *P* value of 0.006 for a 5% risk of making a Type I error. All analyses were made using S-PLUS statistical software (Insightful Corp. Seattle, USA. S-PLUS® 6.0).

Results

Five aliphatic and four indole glucosinolates were found in the plant samples—all nine were present in both foliage and roots. The aliphatic glucosinolates were sinigrin (2-propenyl), gluconapin (3-butenyl), glucoiberin (3-[methylsulphinyl] propyl), progoitrin (2[*R*]-2-hydroxy-3-butenyl), and glucoraphanin (4-[methylsulphinyl] butyl). The indole glucosinolates were glucobrassicin (indol-3-ylmethyl), neoglucobrassicin (1-methoxyindol-3-ylmethyl), 4-hydroxyglucobrassicin (4-hydroxyindol-3-ylmethyl), and 4-methoxyglucobrassicin (4-methoxyindol-3-ylmethyl).

Total glucosinolate concentration of in cabbage foliage did not change in response to treatment, although there was a tendency toward a decrease in glucosinolate concentration both due to egg and clover densities (Table 1). Total root glucosinolate concentration was decreased by clover density, particularly at high egg densities (Table 2). In monocultured control plants, the concentration of foliage glucosinolates was 26±6 μmol g⁻¹ freeze-dried matter (FDM) and in roots

Table 1 Changes in glucosinolate concentrations in cabbage foliage due to *D. floralis* egg density and clover density

	Trivial name	Eggs	Clover	Egg × clover	Egg ²
Aliphatic	Glucoiberin	NS	---	---	---
	Progoitrin	0.03 (-)	0.03 (-)	---	---
	Glucoraphanin	NS	---	---	0.066 (+, -)
	Sinigrin	0.053 (-)	---	---	---
	Gluconapin	0.056 (-)	<0.001 (-)	---	---
	Total Aliphatic	0.059 (-)	---	---	---
Indole	Hydroxyglucobrassicin	---	<0.001 (-)	---	---
	Methoxyglucobrassicin	0.063 (-)	---	---	---
	Glucobrassicin	---	<0.01 (-)	---	---
	Neoglucobrassicin	0.04 (-)	---	---	---
	Total Indole	---	0.018 (-)	---	---
	Total glucosinolates	0.058 (-)	0.053 (-)	---	---

Table presents *P* values, followed by (+) or (-) to describe the changes in concentrations. When nonlinearity was observed (Egg²), + and - are used to describe the appearance of the curve. A *dashed line* represents variables excluded from the analysis when they did not improve the explanatory power of the GLM.

10±0.6 μmol g⁻¹ FDM. The concentrations of individual glucosinolates were generally higher in foliage than in roots, although this was not the case for hydroxyglucobrassicin or for neoglucobrassicin at high egg densities. MANOVA showed that there was an effect of intercropping density on individual glucosinolates for both foliage (approximately *F*=2.70; *d.f.*=2; *P*=0.017) and roots (approximately *F*=4.60; *d.f.*=2; *P*<0.001). Root glucosinolates were also affected by egg density (approximately *F*=5.63; *d.f.*=1; *P*=0.008). GLM analyses suggested that individual glucosinolates responded variously to treatment, even within the aliphatic and indole groups.

Of the foliage glucosinolates, glucobrassicin and aliphatic sinigrin were the dominant compounds in the foliage of monocultured control plants with concentrations of 12±2 and 10±3 μmol g⁻¹ FDM, respectively. Glucobrassicin levels decreased in response to intercropping but were not significantly altered by herbivory. The concentrations were

almost identical for cabbage plants grown with high and low densities of clover and intercropping at the different egg densities and resulted in a decrease in glucobrassicin by nearly half. No statistically significant responses were found for sinigrin, although there was a tendency toward lower concentrations in infested plants. Of the remaining aliphatic glucosinolates, gluconapin concentration decreased sharply in intercropped plants (Table 1). This compound was found in small amounts in monocultured control plants (0.69±0.15 μmol g⁻¹ FDM) and had a mean reduction of 54% in intercropped plants for all egg densities (mean of IC1 and IC2). Progoitrin (2.1±0.35 μmol g⁻¹ FDM in monocultured control plants) was the second most common aliphatic glucosinolate, and the only glucosinolate in the foliage that responded to both egg and clover density—in both cases with a reduction in concentration (Table 1). Glucoiberin and glucoraphanin were not changed by either turnip root fly damage or intercropping, as was the total concentration of

Table 2 Changes in glucosinolate concentrations in cabbage roots in response to *D. floralis* egg density and clover density

	Trivial name	Eggs	Clover	Egg × clover	Egg ²
Aliphatic	Glucoiberin	0.011	<0.001	---	---
	Progoitrin	0.043	<0.001	---	---
	Glucoraphanin	0.007	0.08	---	---
	Sinigrin	<0.001	0.006	0.006	0.041
	Gluconapin	<0.001	0.003	<0.001	<0.001
	Total Aliphatic	<0.001	0.001	0.008	0.018
Indole	Hydroxyglucobrassicin	NS	<0.001	0.004	---
	Glucobrassicin	---	0.003	---	---
	Methoxyglucobrassicin	<0.001	<0.001	---	0.042
	Neoglucobrassicin	<0.001	---	---	0.001
	Total Indole	<0.001	---	---	0.057
	Total glucosinolates	NS	0.004	0.021	---

Table presents *P* values. A *dashed line* represents variables excluded from the analysis when they did not improve the explanatory power of the GLM.

aliphatic glucosinolates (Table 1). The latter was probably influenced by the lack of response by the dominant sinigrin. The concentration of hydroxyglucobrassicin also decreased significantly in intercropped plants. Hydroxyglucobrassicin was a trace glucosinolate component ($0.18 \pm 0.06 \mu\text{mol g}^{-1}$ FDM) in monocultured control plants, and decreased by a mean of 53% in intercropped plants at all egg densities (mean of IC1 and IC2). Neoglucobrassicin, the second most common indole glucosinolate ($0.89 \pm 0.23 \mu\text{mol g}^{-1}$ FDM in monocultured control plants), decreased in concentration in response to increasing egg densities while the concentration of methoxyglucobrassicin remained unchanged ($0.41 \pm 0.15 \mu\text{mol g}^{-1}$ FDM in monocultured control plants). Similar to glucobrassicin, the dominant glucosinolate in foliage, the total concentration of foliage indole glucosinolates responded to treatment with a decrease with clover density (Table 1).

Root glucosinolates responded differently and in a more complex manner than foliar glucosinolates. Sinigrin was the

dominant glucosinolate ($6.8 \pm 0.3 \mu\text{mol g}^{-1}$ FDM in monocultured control plants) in roots. Both monocultured and intercropped plant roots contained similar sinigrin concentrations. However, the concentration of sinigrin decreased with egg density in intercropped plants. In monocultured roots, sinigrin concentration was similar to the levels in control plants at all egg densities except for the ten egg treatments where the concentration was somewhat lower. This mixed response caused both an interaction between eggs and clover and a tendency toward a nonlinear response (Table 2, Fig. 1). Progoitrin, another aliphatic glucosinolate, occurred in relatively large amounts in monocultured control plants ($1.1 \pm 0.2 \mu\text{mol g}^{-1}$ FDM) and decreased in concentration with increasing clover density (Table 2, Fig. 1). Glucoiberin decreased in concentration in response to both egg and clover densities, while glucoraphanin tended to increase as a result of damage by *D. floralis* (Table 2, Fig. 1). Gluconapin showed a strong nonlinear response as it decreased rapidly with egg inoculation at low

Fig. 1 Changes in concentration of individual aliphatic glucosinolates ($\mu\text{mol g}^{-1}$ FDM) in cabbage roots caused by an increase in clover density (IC1 and IC2) compared with monoculture (M), and increasing *D. floralis* egg density (0, 10, 20, and 40 eggs; $N=2$)

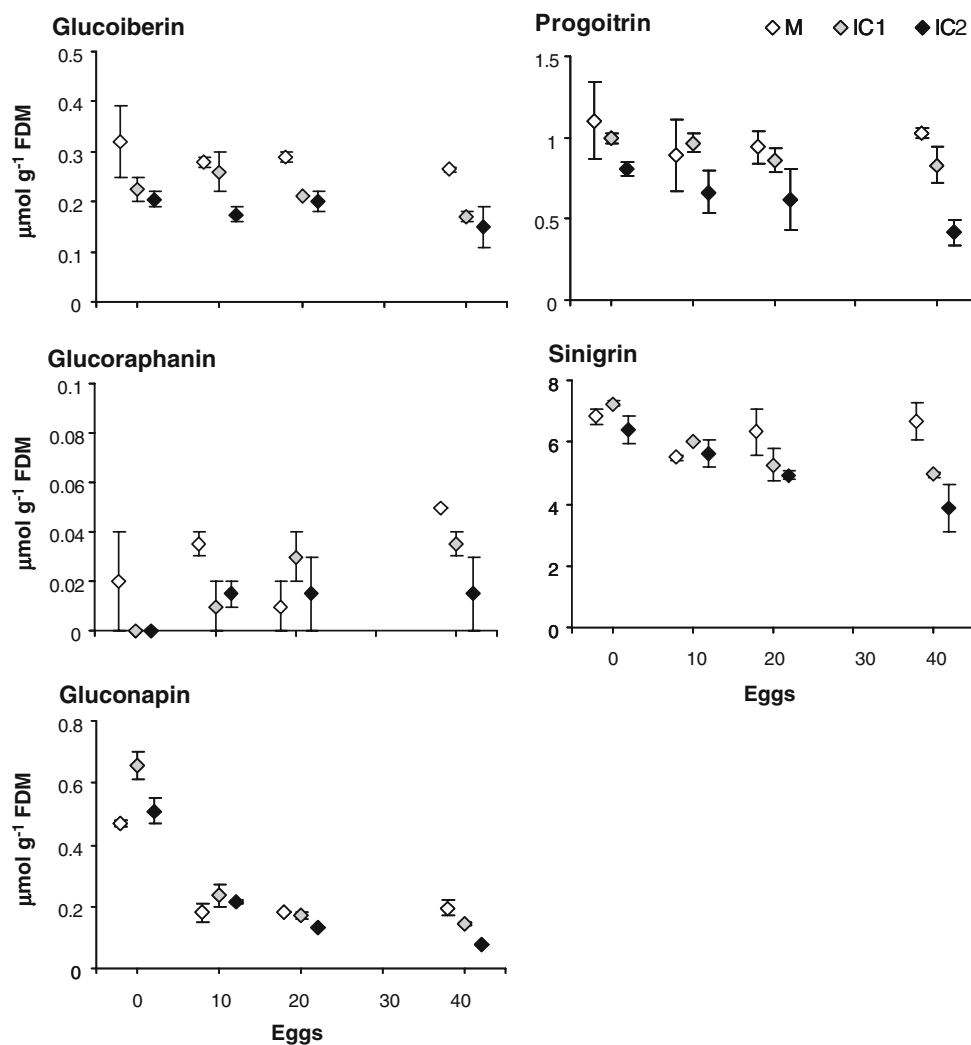
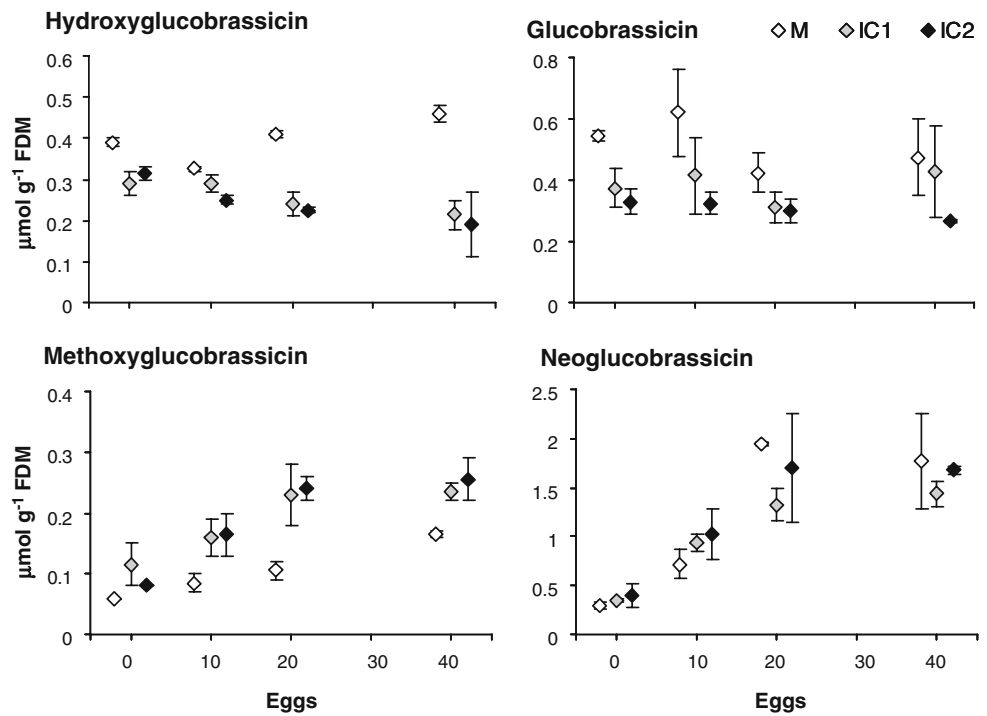


Fig. 2 Changes in concentration of individual indole glucosinolates ($\mu\text{mol g}^{-1}$ FDM) in cabbage roots caused by an increase in clover density (*IC1* and *IC2*) compared with monoculture (*M*), and increasing *D. floralis* egg density (0, 10, 20, and 40 eggs; $N=2$)

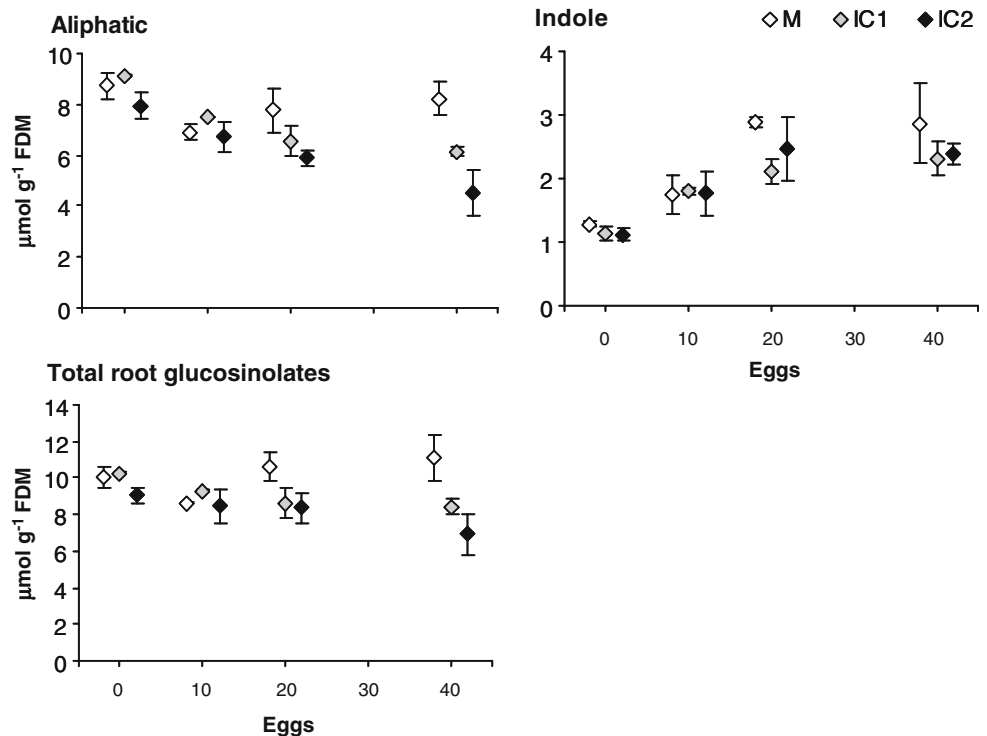


egg densities, while additional eggs did not result in much further decreases (Table 2, Fig. 1). The higher concentrations in intercropped control plants and the lower concentrations in intercropped plants with higher egg densities caused an interaction effect between egg and clover (Table 2, Fig. 1). As the aliphatic glucosinolates were dominated by sinigrin, the response of the group as a

whole was almost identical to that of sinigrin (Table 2, Figs. 1 and 3).

Of the indole glucosinolates found in roots, neoglucobrassicin responded strongly to egg density and concentrations increased from $0.39 \pm 0.01 \mu\text{mol g}^{-1}$ FDM in monocultured control plants to $1.95 \pm 0.03 \mu\text{mol g}^{-1}$ FDM in monocultured plants at a density of 20 eggs.

Fig. 3 Changes in concentration of aliphatic, indole, and total glucosinolates ($\mu\text{mol g}^{-1}$ FDM) in cabbage roots caused by an increase in clover density (*IC1* and *IC2*) compared with monoculture (*M*), and increasing *D. floralis* egg density (0, 10, 20, and 40 eggs; $N=2$)



Additional increases did not occur in plants inoculated with 40 eggs, thus, resulting in a significant nonlinear response in monocultured plants (Table 2, Fig. 2). The concentration of hydroxyglucobrassicin had contrasting patterns in cabbage grown in monoculture vs. intercropping. As hydroxyglucobrassicin concentration decreased with egg density at both clover densities, it increased somewhat in the monoculture (Table 2, Fig. 2). The concentration of glucobrassicin was consistently higher when grown in monoculture (Table 2, Fig. 2). Methoxyglucobrassicin increased with egg density. In addition, the concentrations of this glucosinolate were higher in intercropped plants than in monocultured plants (Table 2, Fig. 2). Neoglucobrassicin and methoxyglucobrassicin were the only glucosinolates in roots to respond to egg inoculation with increased concentrations. This means that neoglucobrassicin had opposite reactions in shoots and roots as this glucosinolate tended to decrease with egg density in the foliage. The total concentration of indole glucosinolates increased in response to egg density similar to the neoglucobrassicin, the dominate glucosinolate (Table 2, Figs. 2 and 3).

Discussion

Earlier studies that examined glucosinolate levels in brassicaceous host plants following herbivory by root flies considered larval damage as the sole stress factor. The responses to larval feeding found previously correspond to herbivore-induced changes that occurred in the present study. Indole glucosinolate levels in roots, particularly neoglucobrassicin and glucobrassicin, tend to increase in response to root herbivory in most studies (Birch et al. 1992, 1996; Griffiths et al. 1994; Hopkins et al. 1998a; van Dam and Raaijmakers 2006). Whether or not these studies showed a net increase in the total amount of root glucosinolates seemed to depend on the relative size of the increase in indole glucosinolates vs. the decrease in aliphatic glucosinolates (Birch et al. 1992; Hopkins et al. 1998a). In our study, damage-induced increases in indole glucosinolates and the corresponding decrease in aliphatic glucosinolates did not balance each other out in intercropped roots (Table 2, Fig. 3).

The lack of response to root damage by total leaf glucosinolate content has previously been shown by Birch et al. (1992), although they reported decreases in foliar indole glucosinolates in several brassica cultivars, as also shown for cabbage in this study. It is clear that glucosinolate responses to root fly larval feeding in roots and leaves vary among plant species and genotype (Birch et al. 1992, 1996), as well as on a temporal scale (Birch et al. 1996). Our glucosinolate sampling was performed 6 weeks after egg inoculation at the time of pupation, when larval damage was

completed. It is likely that the glucosinolate profile of both roots and leaves may have been different at earlier, or later, sampling dates (Bodnaryk 1992; Griffiths et al. 1994; Birch et al. 1996; van Dam and Raaijmakers 2006).

Besides herbivory, an additional stress factor was added in the present student, namely competition with red clover. The competitive environment within the intercropping is likely to have had consequences for the cabbage plants' ability to defend themselves against *D. floralis* larval feeding. The GDB hypothesis suggests that a plant can either grow or allocate resources to defense, depending on resource availability (Stamp et al. 2004). This should lead to reduced concentrations of defense compounds in competition-stressed plants. The ability of a plant to acquire resources for growth or defense is affected not only by competition but also by root damage, since such damage impairs the plant's ability to acquire resources. Decreased concentrations of several glucosinolates in aerial cabbage parts and the strong tendency towards an overall reduction in glucosinolates (Table 1) suggest that competing plants, as well as plants with root herbivory, do not allocate the same resources as unchallenged plants towards sustaining levels of foliar defense compounds. Total root glucosinolate levels were reduced in intercropped treatments but only at higher egg densities, while glucosinolate levels in monoculture treatments were fairly constant. This suggests that plants subjected to high *D. floralis* infestations are not able to allocate resources to maintain glucosinolate root levels if they are also competing with the clover for light and nutrients. Our findings on secondary compounds in relation to plant competition confirm the results of other studies. For example Stamp et al. (2004) found that foliar concentrations of chlorogenic acid, rutin, and tomatine in tomato (*Lycopersicon esculentum*) decreased with increasing competition. Furthermore, Cipollini and Bergelson (2001) reported decreasing nutrient availability through plant competition-mediated, density-dependent reductions in trypsin inhibitor levels of in *B. napus* seedlings. They later showed that this decrease in defense led to higher levels of leaf herbivory in a field situation (Cipollini and Bergelson 2002). From the results of this study, it is clear that within the usual division of glucosinolates into aliphatic, aromatic, and indole-based, that certain glucosinolates may not respond uniformly to herbivory or plant competition. Even if relevant to describe changes in different glucosinolate groups classified according to structure, this is likely to conceal the fact that individual glucosinolates within each group may respond in different manners. For cabbage roots, this was the case for methoxyglucobrassicin, which, in contrast to other indole glucosinolates, increased in intercropped treatments (see Fig. 2). It was also evident that the changes that occur within a glucosinolate group may be strongly influenced by changes in a single compound

(Griffiths et al. 1994; Hopkins et al. 1998a; van Dam and Raaijmakers 2006). In cabbage foliage, only indole glucosinolates responded to treatment with increasing clover density by decreasing the dominating glucobrassicin. The responses of aliphatic and indole glucosinolate groups in roots were dominated by sinigrin and neoglucobrassicin, respectively. Our results confirm the importance of considering nonlinear responses, as they were observed to occur in several instances. Root gluconapin and neoglucobrassicin concentrations had the strongest nonlinear responses. In swede roots (Hopkins et al. 1998a), neoglucobrassicin and gluconapin had nearly identical nonlinear responses to increased egg load as found with cabbage in this study. A nonlinear response of glucobrassicin has also been shown for *B. napus* cotyledons wounded mechanically through an increasing number of needle punctures (Bodnaryk 1992). The additional stress from competition in this study also affected nonlinear responses to herbivory. The interaction between intercropping and egg density for gluconapin in roots could be explained by the nonlinear response to an increased herbivore load being more marked in intercropped roots than for monocultured cabbage.

Both root and leaf herbivories could have negative implications for the subsequent development of specialist leaf herbivores through systemic induced changes in the glucosinolate-myrosinase system in undamaged leaves (van Dam et al. 2005; Martin and Müller 2007). An indication of systemic induction of glucosinolate concentrations in response to larval damage in our study was the minor, but significant, decrease in foliar progoitrin and neoglucobrassicin. The effect of intercropping, which decreased concentrations of gluconapin, hydroxyglucobrassicin, and glucobrassicin in intercropped cabbage foliage, is likely to be a result of both above- and below-ground competition, as intercropped cabbage plants at the end of the experiment were almost overgrown by the clover. Glucobrassicin and gluconapin have been identified as oviposition stimulants for *D. radicum* and *D. floralis* (Roessingh et al. 1992; Simmonds et al. 1994; Gouinguéné and Städler 2006), as well as other specialists, such as *Pieris brassicae* (van Loon et al. 1992). A decrease in the concentration of these compounds in aerial parts resulting from plant competition could lead to less attractive cabbage plants for specialized pest insects in intercropped systems. In contrast, lower leaf glucosinolate levels may have implications on the generalist herbivore community, as infestation levels may increase as defense levels decrease (Cipollini and Bergelson 2002). Changes in the glucosinolate profile of roots by competition and herbivory are most likely important for subsequent *D. floralis* and *D. radicum* females approaching plants for the purpose of oviposition. *D. radicum* females prefer to lay eggs on plants with moderate damage compared with undamaged or heavily infested plants (Baur et al. 1996a),

and cues perceived from the roots of these plants are likely to be of importance for host selection by females (Baur et al. 1996b).

To evaluate the importance of the changes in this and other studies for host seeking and preference of brassica-specialized insects, one needs to investigate how the actual changes are perceived by herbivores. Evaluations of single factors in complex systems are far from easy, as other plant characteristics change at the same time as the concentrations of secondary compounds change, e.g., plant biomass, nutrient and fiber content (Björkman, unpublished data). Adding competition to the study of herbivore-induced glucosinolate responses has revealed complex relationships between responses and causal agents. This shows the importance of investigating the impact of multiple challenges on plant composition and the possible implications for herbivore host plant search and acceptance.

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