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Root and shoot glucosinolates: a comparison of their diversity, function and interactions in natural and managed ecosystems

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Abstract The role of glucosinolates in aboveground plant–insect and plant–pathogen interactions has been studied widely in both natural and managed ecosystems. Fewer studies have considered interactions between root glucosinolates and soil organisms. Similarly, data comparing local and systemic changes in glucosinolate levels after root- and shoot-induction are scarce. An analysis of 74 studies on constitutive root and shoot glucosinolates of 29 plant species showed that overall, roots have higher concentrations and a greater diversity of glucosinolates than shoots. Roots have significantly higher levels of the aromatic 2-phenylethyl glucosinolate, possibly related to the greater effectiveness and toxicity of its hydrolysis products in soil. In shoots, the most dominant indole glucosinolate is indol-3-ylglucosinolate, whereas roots are dominated by its methoxyderivatives. Indole glucosinolates were the most responsive after jasmonate or salicylate induction, but increases after jasmonate induction were most pronounced in the shoot. In general, root glucosinolate levels did not change as strongly as shoot levels. We postulate that roots may rely more on high constitutive levels of glucosinolates, due to the higher and constant pathogen pressure in soil communities. The differences in root and shoot glucosinolate patterns are further discussed in relation to the molecular regulation of glucosinolate biosynthesis, the within-tissue distribution of glucosinolates in the roots, and the use of glucosinolate-containing crops for biofumigation. Comparative studies of tissue-specific biosynthesis and regulation in relation to the biological interactions in aboveground and belowground environments are needed to advance investigations of the evolution and further utilization of glucosinolates in natural and managed ecosystems.

Keywords Allelopathy · Induced responses · Isothiocyanates · Plant–environment interaction · Soil ecology

Abbreviations
GSL Glucosinolates
ITC Isothiocyanates

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Introduction

Glucosinolates (GSL) are a widely studied class of plant chemical compounds with a large structural diversity. Over 120 GSL have been identified to date, mainly in species belonging to the Brassicaceae (Fahey et al. 2001). The large diversity in GSL profiles between and within species has been extensively recorded, especially for various crop cultivars and varieties, and the model plant species *Arabidopsis thaliana*. However, there has been a strong focus on GSL analyses of aboveground plant organs, probably because the main crucifer crops, such as oil seed rape (*Brassica napus*), cabbages and broccoli (*B. oleracae*), have their harvestable parts aboveground. In these crops GSL influence important quality and flavour characteristics of the produce, as well as resistance against non-adapted pathogen and insect pests (Chew 1988; Mithen 2001). Similarly, studies on the induction of GSL mainly focus on the effects of aboveground herbivores or pathogens. Consequently, constitutive and induced GSL levels and profiles in roots are under-explored relative to those of aboveground plant parts (van Dam et al. 2003).

Evolutionary theory predicts that the large diversity of plant defence compounds, such as GSL, reflects the present and past selection pressures experienced by plants in their natural environment (Jones and Firn 1991). Consequently, it has been hypothesized that the vast variety of GSL found in plant species has arisen from an evolutionary arms race with their enemies (Benderoth et al. 2006). Assuming that GSL serve a defensive function in roots as well, we may expect similar natural selection processes to act on belowground GSL composition. However, the kind of selection pressures exerted by soil processes may differ completely from the aboveground processes. First, the physical and chemical environment of roots is completely different than that of aerial parts. This may require different compounds in roots and shoots to serve similar biological functions. Second, the aboveground and belowground communities interacting with the plant differ as well. On the one hand, pressures exerted by soil biota may be more constant, because most agricultural and natural soils are truly 'living soils' full of micro-organisms and nematodes 'waiting' for a plant root to feed on (Coleman et al. 2004). On the other hand, the composition of the soil community interacting with an individual plant may be much more random. Heterotrophic soil organisms are often concentrated in nutrient-rich patches in the soil (Coleman et al. 2004). Moreover, soil biota such as micro-organisms and nematodes are far less mobile than aboveground herbivores and pathogens, which can be transported over longer distances by wind and rain (van der Putten et al. 2001). Consequently, the distribution of soil biota is heterogeneous, so predicting which soil organisms a root will encounter when the seed starts to germinate is difficult. Reasoning along the same evolutionary lines, the induction of root and shoot GSL may differ as well. If roots have a higher risk of exposure to herbivores, it may be beneficial to constitutively produce high levels of GSL (Karban et al. 1999). Although these assumptions are rooted in ecological-evolutionary theory, the same processes may apply to breeding for crop resistance. Artificial selection procedures have successfully been aiming at contrasting selection trajectories for GSL levels in different organs. For example, breeders have been selecting for both lower GSL levels in seeds to improve oil quality for consumption, as well as for higher GSL levels in roots to increase resistance against phytophagous nematodes (Potter et al. 2000), or suppress fungal diseases in following cereals species (Kirkegaard et al. 2001).

Here we comprehensively summarize studies that have analyzed both root and shoot GSL levels in the same plant, and identify differences between the two. Similarly, we summarize data on aboveground and belowground GSL induction. To facilitate the straightforward comparison of induced GSL responses, we focus on those studies using induction hormones instead of a variety of herbivores and pathogens. In addition to local induction processes, we also discuss aboveground belowground interactions between induced responses. Interactions between root- and shoot-induced responses have received increasing interest lately, because they may interfere with local induction of optimal defence responses and significantly affect higher trophic levels associated with plants (Soler et al. 2005; van Dam and Raaijmakers 2006). The differences in root and shoot GSL levels and profiles will be linked to their known functions in pathogen and insect resistance, as well as discussed in the light of the different

physical properties of air and soil. In addition, we discuss how the existing knowledge on the molecular regulation of GSL biosynthesis (reviewed by Gigolashvili et al., this issue; Kliebenstein, this issue) and the within tissue distribution of root GSL will help to understand the observed patterns. Finally, we consider the application of GSL-containing crops for biofumigation in agricultural systems, and identify directions for future research that will help us to increase our understanding of the roles of GSL in natural and managed ecosystems.

Constitutive levels of root and shoot GSL

A comprehensive literature search yielded records of root and shoot GSL levels and profiles of 29 plant (sub)species (Table 1). Not surprisingly, the majority of the records were on members of the Brassicaceae (69 out of 74), mostly cultivated *Brassica* species and varieties (41 of 69). In addition, we also found several records of wild plant species belonging to other families such as the Caricaceae, Moringaceae, Salvadoraceae and Tropaeolaceae (Table 1). Despite a large variation in how the plants were grown, the ontogenetic stage of the plants, the season in which they were harvested, and the detail to which the analyses were performed at the tissue level, we were able to identify several general trends in the dataset. Overall, roots had higher total GSL concentrations than shoots (Appendix 1 root/shoot ratio average, Wilcoxon's matched pairs test, $n = 47$, $Z = 4.98$, $P < 0.001$). Root tissues on average had 4.5 ($\pm$5.6 SD) times more GSL than shoot tissues. Only 15 records reported root levels that were lower than shoot levels (Appendix 1). Three of these records were on flowering *B. nigra* (Kirkegaard and Sarwar 1998; van Dam et al. 2004; Bellostas et al. 2007). In this species, the general rule of a high root/shoot GSL ratio only applies to the vegetative stage; roots of vegetative plants with four leaves had 3.7 times higher GSL levels than their shoots (Bellostas et al. 2007). In broccoli and *Arabidopsis thaliana* plants, on the other hand, it was the sprouts and young rosettes that had a lower root/shoot GSL ratio than later stages of the same species (Appendix 1). Our analysis also showed that roots on average have a greater diversity of GSL than shoots (Table 2). We cannot rule out the possibility that this is due to the higher overall GSL levels in roots, and thus more compounds exceed the HPLC detection limit in root extracts.

In addition to the differences in total GSL levels, we also found significant differences in GSL composition between roots and shoots. Even though the fractions of indole GSL were similar in roots and shoots, there was a distinct difference in the composition of this group. In shoots, the predominant indole GSL is indol-3-ylmethylglucosinolate (I3M; 60%, Table 2), whereas in the roots the 1- and 4-methoxy derivatives dominate (only 23% is I3M). In 30 of the 56 records detailing root GSL profiles, 1-methoxyindol-3-ylmethylglucosinolate (1MI3M) was the most prominent indole GSL in the roots and in 14 cases 4-methoxyindol-3-ylmethylglucosinolate (4MI3M; Appendix 1). Recently it was shown that 1MI3M and 4MI3M and their breakdown products are more potent deterrents of generalist aphid feeding than their precursor I3M (Kim and Jander 2007; Agerbirk et al., this issue). I3M breakdown products, on the other hand, were found to be more effective inhibitors of *Leptospaeria maculans*, a fungus causing stem canker in rapeseed cultures, than those of 1MI3M (Mithen and Lewis 1986). Unfortunately, this class of GSL has so far been little studied for its involvement in resistance against herbivores and pathogens, which makes it difficult to speculate how specific aboveground and belowground processes may have contributed to the observed difference. The lack of attention for indole GSL is probably due to the long-standing assumption that they are less toxic or deterrent than aliphatic GSL. The reason is that they do not yield stable isothiocyanates (ITC) upon contact with myrosinase, but produce the less toxic nitriles and ascorbigens, depending on the presence of modulating proteins such as epithiospecifier protein (ESP) and epithiospecifier modifier 1 protein (ESM1; Agerbirk et al. 1998; Burow et al. 2008).

Another significant difference between root and shoot GSL profiles was the levels of 2-phenylethylglucosinolate (2PE-GSL). Especially in *Brassica* species, 2PE-GSL is often the major GSL in the root profile, whereas it is either absent or found in trace amounts in shoots (Table 2). In the argument for an evolutionary basis for the predominance of 2PE-GSL in roots, it is revealing to consider the comparative advantage of that compound over GSL with other side-chain structures. Firstly, its break-down product

Table 1 Plant species, the number of records (natural or cultivated) per species that were included in our analysis of root and shoot GSL, and the sources for the data

Plant species	Records on natural species	Records on cultivated species	Source references
Alliaria petiolata M. Bieb.) Cavara & Grande	2		Vaughn and Berhow (1999)
Arabidopsis thaliana (L.) Heynh.	5		Petersen et al. (2002) and Brown et al. (2003)
Armoracia rusticana P. Gaertn., B.Mey & Scherb.	1		Li and Kushad (2004)
Azima tetracantha L.	1		Bennett et al. (2004)
Barbarea vulgaris R. Br.	2		van Leur et al. (2006), van Leur (2008) and van Leur et al. (2008)
Brassica campestris L.		1	Kirkegaard and Sarwar (1998)
Brassica carinata A. Braun.		3	Kirkegaard and Sarwar (1998) and Bellostas et al. (2007)
Brassica fruticulosa Cirillo		1	Kirkegaard and Sarwar (1998)
Brassica juncea (L.) Czern.		7	Kirkegaard and Sarwar (1998, 1999) and Bellostas et al. (2007)
Brassica napus L.		10	Birch et al. (1992) and Kirkegaard and Sarwar (1998, 1999)
Brassica nigra (L.) W.D.J. Koch	3	1	Kirkegaard and Sarwar (1998), van Dam et al. (2004) and Bellostas et al. (2007)
Brassica oleracea L.	3	13	Birch et al. (1992), Rosa (1997), Rosa and Rodrigues (1998), Castro et al. (2004), Charron and Sams (2004), Aires et al. (2006) and Gols and Van Dam (unpublished data)
Brassica rapa L.		3	Bellostas et al. (2007) and Smetanska et al. (2007)
Brassica rapa L. subsp *oleifera* DC.		2	Loivamäki et al. (2004)
Cardamine cordifolia A. Gray	1		Rodman and Louda (1984)
Cardamine (Dentaria) diphylla (Michx.)Wood.	1		Feeny and Rosenberry (1982)
Cardamine (Dentaria) maxima (Nutt.)Wood.	1		Feeny and Rosenberry (1982)
Carica papaya L.		1	Ludwig-Müller et al. (1999)
Diplotaxis tenuifolia (L.) DC.		1	Kirkegaard and Sarwar (1998)
Eruca sativa Mill.	1	1	Kirkegaard and Sarwar (1998) and Kim and Ishii (2006)
Moringa oleifera Lam.	1		Bennett et al. (2003)
Moringa stenopetala (Baker f.) Cufodontii	1		Bennett et al. (2003)
Sinapis alba L.		1	Kirkegaard and Sarwar (1998)
Sinapis arvensis L.		1	Kirkegaard and Sarwar (1998)
Sisymbrium orientale L.		1	Kirkegaard and Sarwar (1998)
Thlaspi arvense L.	1		Tolrà et al. (2006)
Thlaspi caerulens J & C. Presl.	1		Tolrà et al. (2001)
Thlaspi praecox Wulfen	1		Tolrà et al. (2006)
Tropaeolum majus L.	1		Ludwig-Müller et al. (1999)
Grand total	27	47	

Original data are provided in the electronic appendix

Table 2 Average number of glucosinolates and the contribution of specific glucosinolates to root and shoot profiles

Parameter scored	Shoot			Root			Statistical analysis			
	n	Mean	Std. dev.	*n*	Mean	Std. dev.	Test	Pairs	*Z*-value	*P*
Number of GSL	63	5.54	2.83	70	6.89	3.36	Sign test	51	3.08	0.002
Fraction indole GSL/total GSL	69	0.21	0.22	70	0.17	0.19	Wilcoxon	69	1.38	0.17
Fraction I3M/indole GSL	57	0.60	0.35	63	0.23	0.23	Wilcoxon	57	5.43	<0.001
Fraction 2PE/total GSL	61	0.06	0.14	61	0.41	0.30	Wilcoxon	61	6.11	<0.001

The contribution of the specific glucosinolates was calculated as a fraction by dividing the concentration of the specific glucosinolate over the total glucosinolate concentration of the same tissue. Abbreviations: n = number of records in which this parameter was quantified; GSL = glucosinolate; I3M = indol-3-ylmethylGSL; 2PE = 2-phenylethyl GSL. Pairs = non-ties/full pairs included in analysis

2-phenylethyl isothiocyanate (2PE-ITC) is among the least volatile (Sarwar et al. 1998), whereas volatile losses are one of the major causes for ITC loss from soil (Brown and Morra 1997). Secondly, 2PE-GSL break-down products are among the most hydrophobic and as a consequence less prone to leaching losses from the soil (Laegdsmand et al. 2007). Thirdly, the aromatic GSL are more lipophilic, which increases membrane permeability, thus contributing to the higher contact toxicity often reported for the aromatic ITCs. Moreover, the ethyl bridge linking the phenyl group with the functional ITC may act to hold the active ITC free of the soil organic matrix allowing better contact with soil organisms (Potter et al. 1998), a structural feature absent from benzyl ITC which is less prevalent in roots (Borek et al. 1998). Even though a recent lab study showed that the volatile insecticidal activity of 2PE-ITC may be mitigated by the soil environment more than that of other ITCs (Matthiessen and Shackleton 2005), many other studies showed that 2-PE ITC was among the most toxic upon direct contact to a range of soil-borne organisms, including soil insects (Borek et al. 1995, 1998), pathogenic fungi (Sarwar et al. 1998), phytophagous root nematodes (Potter et al. 1999, 2000) and wheat seeds (Bialy et al. 1990). 2PE-GSL is also thought to be one of the compounds preventing the association of Brassicaceous plants with arbuscular mycorrhizal fungi (AM). Both agriculturally important as well as wild *Brassica* species are known to be non-mycorrhizal (Harley and Harley 1987). 2-PE GSL was only present in the roots of non-AM hosts, and was absent in the AM host species, such as the non-*Brassica* species *Tropaelum majus* and *Carica papaya.* The roots of these species contained other GSL including the closely related aromatic benzyl-GSL (Vierheilig et al. 2000). Since no plants known to contain 2PE-GSL have been shown to host AM to date, the possibility remains that it may be a potent inhibitor of AM infection. However, the fact that other non-*Brassica* species such as white lupins (*Lupinus alba*) are also non-hosts indicates that mechanisms unrelated to GSL are involved as well.

Experiments with artificially selected canola varieties have shown that it is possible to selectively breed for higher 2PE-GSL levels without affecting shoot or seed GSL levels (Potter et al. 2000; Kirkegaard et al. 2001). These artificial selection experiments suggest that natural selection processes may have contributed to independent selection for GSL levels and profiles in roots and shoots in a similar fashion.

Shoot and root induction of GSL

Even though GSL are present constitutively in all plant tissues (Wittstock and Gershenzon 2002), very often they increase upon herbivore damage, pathogen infection or application of plant hormones. Most of the studies published on the induction of GSL focus on aboveground induction processes. However, several recent studies have shown that root induction may cause both local and systemic changes in GSL levels as well. Root fly feeding, for example, systemically increases 2-propyl GSL levels in the leaves and indole GSL levels in the roots of *Brassica nigra* (Soler et al. 2005; van Dam and Raaijmakers 2006).

Straightforward comparisons between induced responses occurring in roots and shoots are difficult,

because most phytophagous organisms feed specifically on only one of the organs. One way to circumvent this problem is to use induction hormones such as jasmonic acid (JA, or its methylated form MeJA), or salicylic acid (SA/MeSA). These naturally occurring phytohormones can be applied quantitatively to either roots or shoots. When analyzing studies using these hormones to induce cruciferous species, consistent patterns emerge, despite considerable differences in the time-frame of the experiment, and the amount or form in which the induction hormones were applied (Table 3). Jasmonates, for example, are potent inducers of shoot indole GSL. In 17 out of the 20 experiments the shoot indole GSL levels increased significantly after application of jasmonates. This increase was both rapid and long-lasting: in several experiments the indole GSL levels doubled within 1 day after treatment and they stayed 10–20 times higher than in control plants for 14–30 days after induction (Table 3). This considerable increase was jasmonate-specific, since salicylate application generally elicited lower or no increases of indole GSL (Table 3).

Interestingly, in two of the three cases reporting no changes in indole GSL levels, the JA was applied to the roots. Similarly, we found that only shoot JA application consistently increased indole GSL in three ecotypes of *Arabidopsis thaliana*, whereas the same amount of JA added to the roots did not (Fig. 1). The increase of indole GSL biosynthesis after shoot application of jasmonates appears to be very specific, because it was occurring even when there was no significant increase of total GSL levels (KON in Fig. 1). Root JA application, on the other hand, may specifically increase aliphatic GSL levels in the shoot as was observed in a feral *B. oleracea* (Table 3; van Dam et al. 2004). Aliphatic and indole GSL are derived from different pathways that are regulated by specific transcription factors belonging to the Myb family (Gigolashvili et al. 2007a; Hirai et al. 2007). It is as yet unknown which physiological mechanism underlies the organ specific differential induction of these two biosynthetic pathways by root or shoot applied JA. Possibly, JA is conjugated with a different amino acid in roots and shoots, resulting in different signalling cascades and GSL responses (Staswick and Tiryaki 2004).

The aliphatic and aromatic GSL in the shoot did not increase substantially after jasmonate application (Table 3). Both the fold-changes as well as the frequencies with which an increase was reported were less than for the indole GSL. Salicylate application did not increase the levels of these compounds either, which may indicate that these GSL are generally less responsive to the application of induction hormones and possibly other induction events.

As for the constitutive GSL levels, there are fewer reports on root GSL responses than on shoot responses. Although several studies report GSL responses in the roots after JA or SA treatment, roots do not respond as strongly as the shoots within the same plant (Table 3). Root indole GSL, for example, increased in less than half of the experiments quantifying root GSL, even if the JA was applied to the root itself (Table 3). This indicates that the induction of similar root and shoot GSL biosynthetic pathways is differentially regulated, for example by root and shoot specific Myb factors (Czechowski et al. 2004). The relatively low response of the roots after induction, together with the higher constitutive levels in this organ, suggests that roots may have a different optimal defence strategy. As suggested by Karban et al (1999) this may be related to the higher chances of herbivore and pathogen attack belowground.

Because GSL are biosynthetically related, the induced changes in GSL are not independent. Multivariate analyses, such as Principal Component Analysis and Partial Least Squares Regression may reveal correlations between different GSL within changing GSL profiles after induction treatments (J.J. Jansen and N.M. van Dam, unpublished results). Together with gene expression analysis, they may reveal the regulatory network underlying specific GSL responses after root and shoot induction.

Molecular regulation of GSL biosynthesis

The most plausible physiological explanation for the observed differences in root and shoot patterns is that both organs have a different regulation of GSL biosynthesis and turn-over. Transportation of GSL via the phloem over long distances (Chen et al. 2001) is not likely to be the main cause. This is supported by the observation that induction of specific indole GSL by aphids occurs in detached leaves as well, precluding a role for transport from the roots (Kim and Jander 2007). Moreover, GSL metabolism is

Table 3 Changes in the levels of different classes of glucosinolates present in roots and shoots after application of induction hormones

Plant species	Treatment	Time	Changes in shoot levels per class			Changes in root levels per class			Reference
			Indole	Aliphatic	Aromatic	Indole	Aliphatic	Aromatic	
Jasmonate induction									
Arabidopsis thaliana 8 ecotypes	MeJA to leaves	1–2 days	0–2× up	Some up, some down	–	–	–	–	Kliebenstein et al. (2002)
Arabidopsis thaliana Col-0	JA to leaves (3x)	7 days	2× up	No change	–	–	–	–	Mewis et al. (2006)
Arabidopsis thaliana Col-0	MeJA to leaves	0.5 h–1 day	2× up	No change	No change	–	–	–	Jost et al. (2005)
Arabidopsis thaliana Col-0 and Ler	MeJA to leaves	1 day	3–10× up	2–4× up (1 compound)	–	–	–	–	Mikkelsen et al. (2003)
Barbarea vulgaris	JA to leaves	7 days	1.4× up	–	1.4× up	No change	–	1.4× up	van Leur et al. (2006) and N.M. van Dam (unpublished)
Barbarea vulgaris	JA to root	7 days	No change	–	1.4× up	No change	–	1.4× up	van Leur et al. (2006) and N.M. van Dam (unpublished)
Brassica campestris ssp. *pekinensis*	JA to leaves (8×)	9 days	3–5× up	1.5–2× up	0–20× up	2–3× up	0.8× down–8× up	>10× up	Ludwig-Müller et al. (1997)
Brassica juncea	MeJA to leaves	1 day	2× up	No change	–	–	–	–	Bodnaryk (1994)
Brassica napus	MeJA to leaves	7 days	20× up	No change	No change	–	–	–	Doughty et al. (1995)
Brassica napus	MeJA/JA to leaves	1 h–14 days	10–20× up	–	–	–	–	–	Bodnaryk (1994)
Brassica napus (oilseed)	JA to leaves	4 days	8.8× up	No change	–	–	–	–	Bartlet et al. (1999)
Brassica nigra	JA to leaves	7 days	No change	1.4× up	2× up	No change	No change	1.4× up	van Dam et al. (2004)
Brassica nigra	JA to root	7 days	No change	1.5× up	1.4× up	No change	No change	1.4× up	van Dam et al. (2004)
Brassica oleracea	JA to leaves	7 days	10× up	1.6× up	No change	1.5× up	1.5× up	No change	van Dam et al. (2004)
Brassica oleracea	JA to root	7 days	2–3× up	2–3× up	No change	No change	1.3× up	No change	van Dam et al. (2004)
Brassica rapa	MeJA to leaves	1 day	10× up	–	–	–	–	–	Bodnaryk (1994)

Table 3 continued

Plant species	Treatment	Time	Changes in shoot levels per class			Changes in root levels per class			Reference
			Indole	Aliphatic	Aromatic	Indole	Aliphatic	Aromatic	
Brassica rapa ssp *oleifera*	MeJA to leaves	3–7 days	10–100× up	4× up (1 compound)	5× down	1.5–10× up	No change	No change	Loivamäki et al. (2004)
Brassica rapa var *rapa*	MeJA to leaves	12 h–14 days	2–100× up	–	–	–	–	–	Liang et al. (2006)
Brassica rapa var Teltow (turnip)	JA to root	30 days	2× up	No change	Up	1.5× up	0.7× down	2–5× up	Smetanska et al. (2007)
Isatis tinctoria	JA to leaves	2 days	3–7× up	–	–	–	–	–	Galletti et al. (2006)
Sinapis alba	MeJA to leaves	1 day	–	–	No change	–	–	–	Bodnaryk (1994)
Salicylate induction									
Arabidopsis thaliana Ler	SA to shoot	1–2 days	4MeOH 2× up	No change	–				Kliebenstein et al. (2002)
Barbarea vulgaris	SA to leaves	7 days	No change	–	No change	No change	–	No change	N.M. van Dam (unpublished)
Barbarea vulgaris	SA to root	7 days	0.7× down	–	No change	No change	–	No change	N.M. van Dam (unpublished)
Brassica campestris ssp. *pekinensis*	SA to leaves (8×)	9 days	No change	No change	No change	3–4× up	0–3× up	2–3× up	Ludwig-Müller et al. (1997)
Brassica napus	SA soil drench	7 days	2× up	Some up, some down	4× up	–	–	–	Kiddle et al. (1994)
Brassica napus	SA to leaves	1 day	No change	–	–	–	–	–	Bodnaryk (1994)
Brassica nigra	SA to leaves	7 days	No change	No change	No change	No change	No change	No change	van Dam et al. (2004)
Brassica nigra	SA to root	7 days	No change	No change	No change	No change	No change	No change	van Dam et al. (2004)
Brassica oleracea	SA to leaves	7 days	No change	No change	No change	0.8× down	0.8× down	–	van Dam et al. (2004)
Brassica oleracea	SA to root	7 days	No change	No change	No change	0.7× down	0.7× down	0.5× down	van Dam et al. (2004)
Brassica rapa var Teltow (turnip)	SA to root	30 days	1.5× up	No change	Up	2.5× up	0.5× down	2–5× up	Smetanska et al. (2007)

Presented are the average fold changes of the levels compared to control plants. Time = time between induction and harvest/analysis; JA = jasmonic acid; MeJA = methyl jasmonate; SA = salicylic acid; – = no data reported in reference; "no change" = no statistically significant changes reported

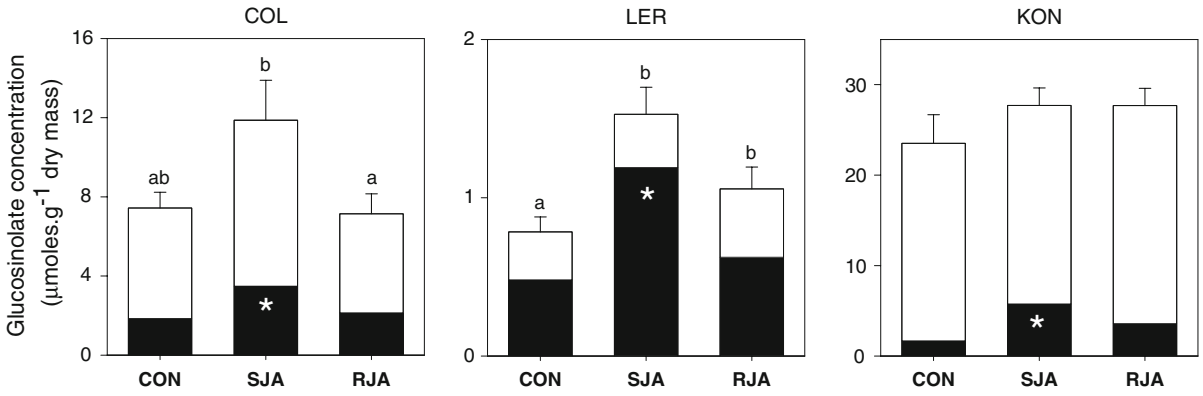

Fig. 1 Indole, aliphatic and total GSL levels (bars are SEM of total levels) of *Arabidopsis thaliana* shoots 7 days after induction with 50 μg jasmonic acid to the shoots (SJA) or to the roots (RJA). Control (CON) plants were treated with equal amounts of acidic water. COL = Columbia ecotype ($n = 10$ per treatment), Ler = Landsberg erecta ($n = 6$), KON = Kondara ($n = 6$). Different letters over bars indicate significant difference in total GSL levels (Tukey HSD post-hoc analysis after ANOVA). The stars in the indole GSL bars indicate significant differences in indole GSL levels between the SJA treatment and the other two treatments

highly integrated with plant growth. For example, there is a tight direct link between indole GSL biosynthesis and the principal auxin, indol-3-acetic acid (IAA) metabolism; they are both derived from tryptophan and they share the first dedicated step in their biosynthesis (reviewed in Grubb and Abel 2006). IAA is a key regulator in plant development and tissue differentiation processes (De Smet and Jürgens 2007). Consequently, many GSL mutants and over-expressing transformants show severe morphological phenotypes, such as increased root branching or stunted shoot growth (Skirycz et al. 2006; Gigolashvili et al. 2007a). In turn, this also implies that environmental factors, both biotic and abiotic, affecting IAA-regulated changes in growth rate or shoot/root ratios, may affect the levels of indole GSL. Additionally, GSL biosynthesis may also be interfering with defence signalling pathways. Increased accumulation of aromatic GSL was shown to stimulate SA-mediated defenses, while suppressing JA-dependent defenses (Brader et al. 2006). Hence the question of what makes the GSL profile different between roots and shoots may be intimately associated with the physiological differences between root and shoot metabolism in general.

Recently, much progress has been made in identifying transcription factors of GSL biosynthesis (Yan and Chen 2007; Gigolashvili et al., this issue). Several of these GSL transcription factors showed organ specific expression patterns (Gigolashvili et al. 2007a, b, 2008). However, a more detailed analysis of the tissue specific regulation of GSL synthesis and turn-over, as well as integration into the general metabolism is needed to elucidate the extent to which differential expression of these genes is responsible for the differences that emerged from our meta-analysis.

Tissue-specific distribution of the GSL-myrosinase system in roots

The tissue-specific distribution of the GSL-myrosinase system can provide some clues as to its likely mode of action, but again there is a dearth of information for roots of field-grown plants. In aboveground tissues, the defensive mode of action against generalist herbivores, particularly during seedling recruitment, is associated with a concentration of GSL in young, growing tissues and reproductive organs (Petersen et al. 2002; Brown et al. 2003; Lambdon and Hassall 2005). Whether the within root distribution of GSL follows similar rules is an open issue. A quick survey of the few data available shows that among five *Brassica* species, there may be consistent allocation patterns within roots as well (Table 4). When ranked within species, primary and lateral roots had the highest levels of GSL and a larger fraction of 2PE-GSL (Table 4; Kruskall–Wallis analysis on ranks, total GSL: $P = 0.0186$, 2PE-GSL

Table 4 Average within root glucosinolate levels (μmol g^{-1} dry mass; standard deviation between brackets) of 5 *Brassica* species: *B. juncea* (2 varieties) and *B. napus* (2 varieties; Kirkegaard and Sarwar 1999), *B. rapa* (Smetanska et al. 2007), *B. nigra* (flowering and rosettes; van Dam and Raaijmakers 2006; Soler et al. 2007), feral *B. oleracea* (van Dam and Raaijmakers 2006)

Root type	*n*	Aliphatic GSL	Aromatic GSL	Indole GSL	Total GSL	2PE/total	Indole/total
Fine/secondary	8	2.9 (3.3)	3.7 (3.3)	3.6 (2.3)	10.2 (7.6)	0.31 (0.14)	0.37 (0.22)
Lateral	4	8.1 (11.0)	17.3 (13.5)	2.5 (1.5)	27.9 (25.0)	0.62 (0.12)	0.12 (0.11)
Tap/primary	8	10.0 (12.5)	9.5 (9.2)	1.4 (1.2)	20.9 (22.1)	0.43 (0.20)	0.10 (0.12)

2PE = 2-phenylethyl GSL; GSL = glucosinolates

fraction $P = 0.018$). The fine roots, on the other hand, showed a significantly larger proportion of indole GSL (Table 4, Kruskall–Wallis on ranks, $P = 0.0034$), which may be due to the fact that actively growing root tips produce high levels of the biosynthetically closely related IAA (Grieneisen et al. 2007).

We know of only one recent study where GSL have been investigated in separated portions of individual roots and quantified in individual cells (McCully et al. 2008). In this study, intact canola roots were cryo-fixed and the vacuoles of individual cells were targeted for elemental sulphur analysis using X-ray microanalysis (EDX) while the specimens were observed under a cryo-scanning electron microscope (SEM). The quantitative cryo-analytical analysis with a SEM showed that the highest concentrations of GSL were found in two cell layers just under the outermost layer of roots with secondary growth. Up to 100× the published GSL concentrations for whole roots were determined for individual cells in these peripheral layers (Fig. 2). Cells of primary tissues had negligible GSL levels. Myrosinase idioblasts, on the other hand, were confined to secondary phloem and inner pericycle. The authors conclude that gross mechanical damage to the mature roots would allow ITC release, whereas less invasive damage may not. GSL, however, would be released continuously to the rhizosphere as roots expand circumferentially. Hydrolysis would occur either from myrosinase distributed within the peripheral cells layers of the root or in the rhizosphere. Based on this distribution of the GSL-myrosinase system in field grown *B. napus* roots, McCully et al. (2008) speculate that the major defensive role in these mature plants appears to be related to the protection of large roots during the critical seed filling stages when these roots are acting as pipelines for nutrients and water absorbed by the fine roots. The same authors suggest that the root-rot fungus *L. maculans*, which first infects the leaves and enters the root via the xylem, may be confined to the vascular bundle by this ring of cells containing high levels of 2PE-GSL (Sprague et al. 2007). The fine roots need less protection, since they are ephemeral and continuously replaced from meristems located in the outer regions of thickened roots (Table 4; M. McCully, personal communication). Such observations are consistent with the previous reports of low concentrations of GSL and ITCs (Rumberger and Marschner 2004) in the rhizosphere of intact growing *Brassica* plants. As the persistence of ITC released in the soil is generally short-lived (1–5 days; Brown and Morra 1997), such a system

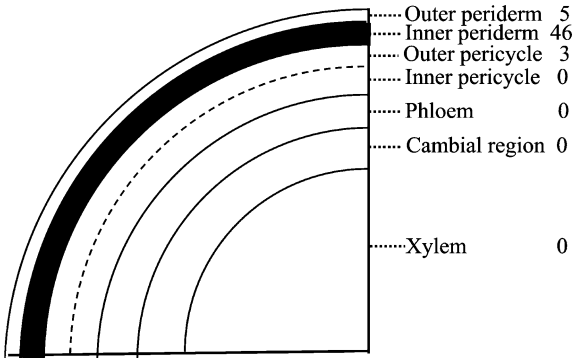

Fig. 2 Schematic of a cross section of a field-grown canola root at early flower development. Numbers indicate glusocinolate concentrations (mM) in individual cells of secondary tissues (including proliferated pericycle), determined by quantitative, cell-specific cryo-analytical analysis of [S] (see McCully et al. 2008). The numbers are mean values for all cells analysed in each tissue region. GSL concentrations of individual cells ranged fairly widely in the three outer tissues, but many more cells with high concentrations were consistently found in the inner periderm (up to 200 mM; shown as dark band). The mean GSL concentration of the top 25% of inner periderm cells analysed was 103 mM ($n = 96$), and 32 mM ($n = 71$), 34 mM ($n = 65$), and 10 mM ($n = 168$) for cells of the outer periderm, and outer and inner pericycle, respectively. Cells of the phloem, cambial region and xylem ($n = 80$, 35, 42, respectively) had no quantifiable glucosinolates

would ensure both protection of the main root system from major disruption as well as a continuous production and release of GSL into the rhizosphere.

Evidence for GSL as mediators for belowground organisms in planta

Despite several studies demonstrating the toxicity of various GSL hydrolysis products to a range of plant pests and pathogens in vitro (e.g. Lazzeri et al. 1993; Serra et al. 2002), there are few clear examples of resistance mechanisms in Brassicacea related to root GSL in planta. Although there are far more studies on the roles of GSL between shoots and aboveground organisms, there are some lines of evidence root GSL may similarly affect both specialist and generalist soil-dwelling pathogens, nematodes and insects. These effects are not always straightforward or direct. For example, it was speculated that GSL were involved in resistance of *B. oleracea* vegetables to the specialist clubroot pathogen *Plasmodiophora brassicacae*. However, it was found that higher root concentrations of indolyl (and possibly aromatic) GSL favour clubroot infection, so that resistant varieties tend to have lower root concentrations of these GSL (Ludwig-Müller et al. 1997). This is thought to be associated with the conversion of the indolyl GSL to IAA and its role in the gall formation associated with the disease.

Lab studies convincingly show that various GSL hydrolysis products are toxic to plant parasitic nematodes (Lazzeri et al. 1993; Serra et al. 2002). There is some support for these studies from field plants as well. It was demonstrated that reduced hosting of *Pratylenchus neglectus* in canola (*B. napus*) lines selected for higher concentrations of 2-PE GSL while there was no correlation with total or other GSL (Potter et al. 1998, 1999). Other non-GSL mechanisms were also involved as many plants with low numbers of *P. neglectus*, had low concentrations of 2PE-GSL (<3 μmol g^{-1}) (Potter et al. 1999). Interestingly, a closely related nematode *P. thornei* which can occur in the same region does not host effectively on canola at all (J.A. Kirkegaard, unpublished data). Variation in the ability of different nematode species to invade and multiply within *Brassica* roots may be due to variations in feeding patterns of the nematodes, and hence variation in their exposure to the GSL system. This may explain why GSL (including 2PE-GSL) were found not to be involved in the reduced host status (invasion, egg laying or development) of a range of Brassicaceous crop plants to the root-knot nematode *Meloidogyne javanica* (McLeod et al. 2001). In contrast to the mobile migratory endoparasitic *Pratylenchus* spp., *Meloidogyne* spp. become sedentary after infection and actively suppress plant resistance responses to form a feeding structure. Consequently, there may be little release of GSL hydrolysis products. Despite the limited evidence for a direct relationship between root GSL content and nematode parasitism, specific use of suppressive impacts of *Brassica* in rotation remains an active research area.

Similarly, there is little evidence that GSL affect root feeding insects. Resistance or susceptibility to attack by the specialist turnip root fly (*Delia floralis*) was not found to be linked to total or individual GSL content, although the relatively high concentration of 2 propenyl GSL was thought to have adversely affected larval feeding and development (Birch et al. 1992). More recently, however, it was found that a related root fly species, *D. radicum*, produced larger pupae on *Barbarea vulgaris* roots with glucobarbarin (*S* 2-OH-2-phenylethyl GSL) as the major GSL than on *B. vulgaris* plants with mainly 2PE-GSL (van Leur et al. 2008).

In addition to these effects on heterotrophic organisms, GSL have also been studied for their allopathic effects on seed germination and plant–plant competition. Observations that certain GSL-containing plants can invade, colonise and dominate some natural ecosystems have led to investigations of the possible role of GSL in these allelopathic interactions. This topic is reviewed elsewhere in this issue (Müller, this issue). Interestingly, the ecological studies aiming at quantifying costs and benefits of GSL, including allelopathic properties of these compounds and their role in plant competition, generally only quantify aboveground GSL levels (e.g. Siemens et al. 2002; Lankau and Strauss 2007), thereby ignoring the role of root GSL in allelopathy.

Use of GSL in agriculture: biofumigation

In agriculture, attempts to harness the biocidal properties of GSL-containing plants have principally

focused on the use of crop rotation, green manure crops or seed meals amended to soil (reviewed by Brown and Morra 1997; Gimsing and Kirkegaard, this issue). These studies have revealed significant potential for suppression of soil-borne pathogens and weed seed germination, although a wide variety of mechanisms unrelated to GSL are also operating, and are often inadequately separated from GSL-related suppression in field studies (Matthiessen and Kirkegaard 2006). Specific targeted investigations of the suppressive potential of root GSL were initiated in Australia during investigations of superior cereal growth following canola (*B. napus*) and mustard (*B. juncea*) in broad-acre farms (Kirkegaard et al. 1994). The dominant GSL in the roots, 2PE-GSL was shown to be highly toxic in vitro to the major soil-borne pathogens of cereals (Sarwar et al. 1998), and canola varieties with higher levels of 2-PE GSL caused a greater reduction in the level of inoculum of the take-all fungus (*Gaeumannomyces graminis*) in pot and field experiments (Kirkegaard et al. 2000). Subsequent studies revealed that such suppression was often not evident as disease reductions in following crops (Smith et al. 2004), partly due to the relatively low concentrations of 2PE-ITC ($\sim$1 nmol g^{-1} soil) which have been measured in the rhizosphere of growing plants (Rumberger and Marschner 2003, 2004). An example is the reported increase in levels of the biocontrol fungus *Trichoderma* spp. in canola rhizospheres (Kirkegaard et al. 2004), which has been shown to be highly tolerant of 2PE-ITC in vitro (Smith and Kirkegaard 2002).

A much wider scope to utilize GSL-containing plants exists in horticultural systems where a variety of species can be utilized as green manures and the whole plant can be incorporated at selected times to maximize the GSL hydrolysis products released (Matthiessen and Kirkegaard 2006). In this context, there has been a stronger emphasis on shoot GSL for several reasons. Firstly in a wide screen of 76 entries, roots contributed only 24% of total plant GSL at the flowering stage (range 2–81%) due to their lower biomass (Kirkegaard and Sarwar 1998). In addition, the more volatile aliphatic GSL, such as 2-propenyl GSL, which dominate the shoot material in many mustard species selected for biofumigation (*B. juncea*, *B. nigra*, *B. carinata*), are considered to better mimic the action of synthetic ITC-based soil fumigants, such as methyl-ITC, because the higher volatility enhanced movement through the soil, and less inactivation of volatile activity (Matthiessen and Shackleton 2005). An obvious difference from an evolutionary perspective is that roots are in continual contact with the soil and likely to be releasing constant low concentrations of GSL into the soil ($\sim$1 nmol g^{-1}), whereas pest control using soil fumigants relies on single high doses (up to 1,500 nmol g^{-1}). In this context a focus on shoot-based ITC for biofumigation may be appropriate.

Similarly, GSL containing plants may be grown to reduce insect pest populations. In New Zealand, the Australian soldier fly (*Inopus rubriceps*) is an important pest as larval feeding on roots can devastate grass pastures. Control has relied on either cultivation measures or insecticide added to the seed. 2PE-ITC isolated from the roots of fodder kale (*B. oleracae*) was found to be insecticidal to soldier fly larvae (Lowe et al. 1971). Similarly, kale or fodder radish (*Raphanus sativus*) sown directly into infected pastures could provide control similar to that achieved with insecticides (76–86% reduction; Blank et al. 1982). The authors suggest that GSL hydrolysis products from roots may have either insecticidal or antifeedant effects. The effectiveness of GSL containing crops as biofumigants may be increased by selecting for higher levels of the effective GSL (Kirkegaard et al. 2001). Additionally, the GSL contents and the effectiveness of weed suppression may be increased by inducing the standing crop by mechanical damage 2 weeks before it is incorporated in the soil (Kruidhof et al. 2008).

Conclusions

By reviewing the current literature on constitutive levels as well as induction of GSL in roots and shoots, we have been able to identify some general patterns that may help us to understand better the role of these compounds in natural and managed systems. Clearly, the levels, distribution and biosynthesis of GSL have been much better defined for the aboveground than for the belowground plant parts. Due to this focus on aboveground plant parts, we may be literally blind to half the story regarding the ecological and agronomical importance of GSL. We therefore argue that more effort should be going into analyzing the belowground GSL profiles and their

role in soil ecological processes. It would provide us with a more profound insight into possible evolutionary and ecological mechanisms that have shaped the observed diversity in GSL profiles. In addition, it would greatly benefit plant breeders wishing to manipulate GSL composition of crop species in a tissue or developmentally specific manner.

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